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Animal Morphology and Physiology

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EDITORIAL

" The Journal of Animal Morphology and Physiology " is now two years old. The zoological world has gratifyingly supported it.

We take this opportunity to express our gratitude to all those who have contributed towards its success. We like particularly to thank The Maharaja Sayajirao University of Baroda, Sri N. J. Bhatt and also the several zoologists who have encouraged it by their generous donations.

The " Journal " has now been made the organ of the Society of Animal Morphologists and Physiologists.

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THE MYOLOGY OF THE CHELONIAN TRUNK AND TAIL

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THE Chelonian is popularly called a "reptile in a box" owing to the presence of the rigid shell which has made free movements of the body wall impossible and thereby rendering the body wall useless for the respiratory movements that are so characteristic of amniotes. These structural modifications in the Chelonia have created a difficult situation for zoologists to explain the mechanism of respiration in these animals. The movements of the neck and limbs are believed to be indirectly serving the purpose of the body wall movements. In our two earlier papers (1954_a and $'55_a$) we dealt with the myology and the movements of the head and neck of the common Indian pond turtle, Lissemys, and the tortoise, Testudo, respectively. In two other papers (1954_b and $'55_b$) we described the striated muscle sheath present on the lung of *Lissemys punctata and Geomyda trijuga* which enables the lungs to function as a pair of bellows and proposed that such might be universally present in the Chelonia. Those studies have encouraged us to make the present study on the comparative myology of the trunk and tail of some of the common chelonians of this country viz., *Lissemys punctata*, *Geomyda trijuga*, *Trionyx gangeticus* and *Testudo elegans*.

Description of the Muscles and Discussion

Muscles of the Trunk : (Figs. 1 & 2)

The epaxial and hypaxial muscles of the trunk in Chelonia are obviously much reduced and modified owing to the fusion of the vertebræ with the carapace resulting in the complete cessation of intervertebral movements, and the formation of the plastron.

The *spinalis-semispinalis* system of muscles in Lissemys (Fig. 1) arises by slender slips from the neural plates and the neural arches of the second to the tenth trunk vertebræ and sometimes also from the first sacral vertebra, which follows the tenth trunk vertebra. Those slips arising from the neural arches of the sixth to the tenth trunk vertebræ and their corresponding neural plates are tendinous, but those arising from the second to the fifth trunk vertebræ and their corresponding neural plates are fleshy. The fibres of all the muscle slips from the second to the tenth trunk vertebræ join together and form a single muscle belly. As in other chelonians this muscle system in Lissemys shows no distinction into its component parts, nor the typical criss-cross arrangement of the tendons characteristic of other reptiles, but consists of a linear column of muscle running through a narrow space bounded by the carapace, ribs and the vertebræ. On emerging out of the space at the level of the first trunk vertebra it gets inserted by a thick tendon on the neural arch and the posterior zygapophysis of the last cervical (8th) vertebra. The muscle acts as an elevator of the neck at the level of the eighth cervical vertebra.

In Trionyx too the *spinalis-semispinalis* system (Fig. 1) has a similar origin, course and insertion, but in Testudo (Fig. 1) the muscle slips arise only from the neural plates covering the first and second trunk vertebræ and also from the neural arches of the same vertebræ. The muscle after passing through the space bounded by the ribs and carapace, emerges out at the anterior end of the first trunk vertebra and gets inserted by a tendon on the neural arch and the

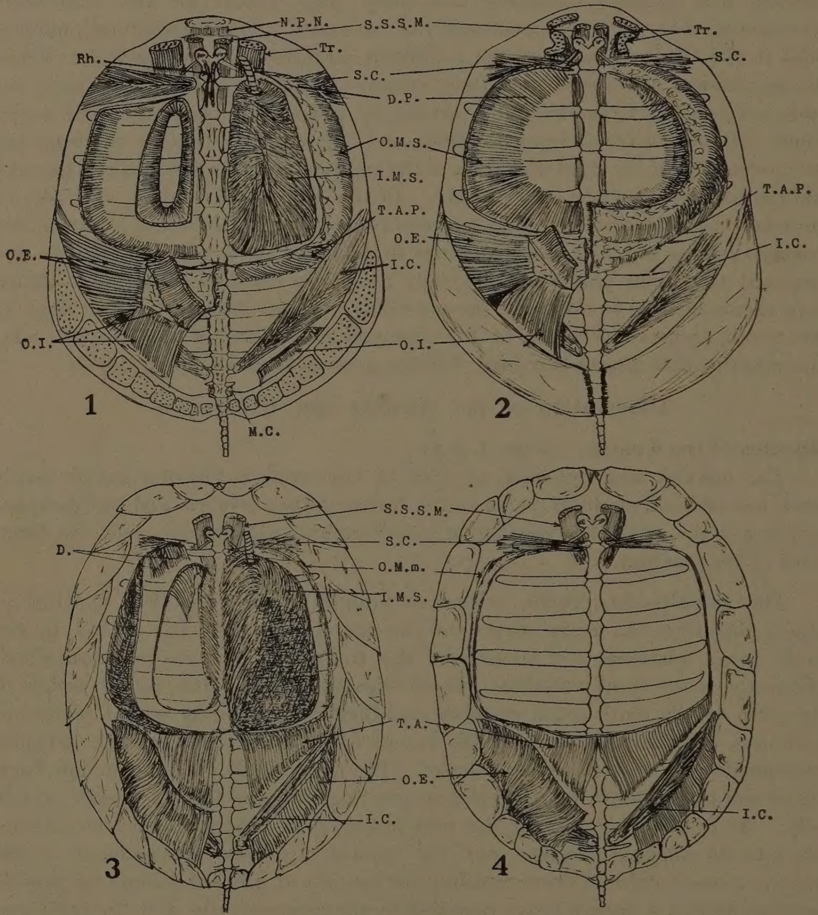

FIG. 1. Ventral view showing the Flank Cavity Muscles : 1, Lissemys ; 2, Trionyx ; 3. Geomyda ; 4. Testudo.

D., diaphragmaticus ; *D.P.*, diaphragmaticus part of the outer muscle sheath ; *I.C.*, iliocaudalis ; *I.M.S.*, inner muscle sheath ; *M.C.*, margino-infra-caudal muscle ; *N.P.N.*, nuchoprenuchalis ; *O.E.*, obliquus externus ; *O.I.*, obliquus internus ; *O.M.m.*, membranous part corresponding to the outer muscle sheath ; *O.M.S.*, outer muscle sheath ; *Rh.*, rhomboideus ; *S.C.*, subclavius ; *S.S.S.M.*, spinalis-semispinalis system of muscles ; *T.A.*, transversus abdominis ; *T.A.P.* transversus abdominis part of the outer muscle sheath ; *Tr.*, trapezius.

posterior zygapophysis of the eighth cervical vertebra. The carapace in Testudo and to a lesser extent in Geomyda, having acquired the shape of a dome and the neck having undergone an unextendable bend at about the level of the first trunk vertebra and thereby become unable to be fully extended as in Lissemys and Trionyx, a great reduction in the *spinalis-semispinalis* system of muscles in the trunk, inevitably resulted. The versatile movements of the neck as in Lissemys, Trionyx and other aquatic and amphibious forms are an absolute necessity for their movements in water, and on land for righting themselves when turned turtle. Testudo on the other hand is unable to right itself when turned turtle and this unfortunate condition is primarily due to the neck having undergone the abrupt bend as a result of the animal acquiring the massive dome-shaped carapace that is a hindrance in swimming. Geomyda (Fig. 1) has only a slightly dome-shaped carapace and has a comparatively small bend at the neck, since only relatively a few vertebræ are involved in the bend and so it is able to right itself only with very great difficulty when turned upside down. This leads us to the inference that in the evolution of the land Chelonia the need to protect themselves from desiccation and other vagaries of an arid environment and to withstand the attack of enemies by taking shelter under cover of a strong armour was a major factor. The trend was naturally towards the aquisition of an extremely hard and heavy shell that could house the whole animal within. Both these ends were achieved in the completely terrestrial forms like Testudo. But all the same they were left behind with a shorter neck with restricted movements and shorter limbs with slower movements as the price for protection. The others on the other hand took to life in water and came to land only occasionally.

In Lissemys there is a small muscle which arises from the anterior border of the nuchal plate and gets inserted on the posterior border and the posterior part of the ventral surface of the prenuchal plate. The muscle when contracts, pulls the prenuchal which is loosely attached to the nuchal, effecting the closure of the shell in front. The muscle (Fig. 1) which has been named as *nuco-prenuchalis* by Hassan (1941) should correspond to the *spinalis* muscle of the seventh and eighth cervical vertebræ, which have lost their usual attachment and taken a position described above. Such a muscle is not seen in Trionyx, in Testudo or in Geomyda, since there is no prenuchal in their carapace.

The *nucho-epiplastralis* (Fig. 1) in Lissemys (Hassan, 1941), corresponds to the *trapezius* which is a well-developed muscle in this animal. It arises from the outer side of the body of the first and second trunk vertebræ and also from the adjoining borders of the nuchal and the first costal plate. The muscle arises as a flat fleshy belly, the fibres of which converge to form a cylindrical mass which in turn curls upon itself and most of the fibres get inserted on the epiplastron, while a few get inserted on the cartilaginous epi-precoracoid. At the level between the last cervical and first trunk vertebræ the cylindrical part of the muscle appears as a pillar on the neck. In Trionyx this muscle is similar in its attachments to that of Lisssemys but differs slightly in having an additional part which arises from the anterior border of the thick fold of skin lining the nuchal plate and bends with the main muscle mass. In the spiny-tailed lizard, Uromastix, this muscle arises from the dorsal fascia and is inserted on the acromio-clavicular joint (George, 1948). The insertion of the muscle in Uromastix is essentially the same as that in Lissemys and Trionyx, when we regard the region of the epiplastron of the plastron as corresponding to the clavicle. So the *mucho-epiplastralis* of Hassan could be regarded as homologous to the *trapezius*. This muscle on contraction ensures the closure of the anterior plastral valve after the retraction of the head and neck into the shell. In Lissemys this valve completely closes the anterior part of the shell, but it is not so complete in Trionyx, while in Testudo and Geomyda it is absent.

The *rhomboideus* (Fig. 1) in Lissemys arises as a flat muscle sheath from the lateral border of the first two trunk vertebræ and also from the fascia of the *trapezius* at its posterior side. The origin of the muscle is fleshy and all the fibres run vertically downwards, where they get inserted on the medial border of the scapula and the dorsal surface of the precoracoid or the acromion process. A few fibres, however, get inserted on the epiplastral cartilage also. The insertion of this muscle on the *median* border of the scapula is similar to that of the mammalian *rhomboideus.* The *rhomboideus* in mammals could be considered as having split off from the well-developed reptilian *trapezius* and in Lissemys too such a splitting of the primitive reptilian *trapezius* seems to have taken place. On contraction the *rhomboideus* pulls the scapula and the precoracoid medially and slightly anteriorly. This retraction of the girdle is needed in order to carry the limbs almost completely under cover of the carapace. No such muscle is present in Trionyx, Testudo or Geomyda.

Regarding the mechanism of respiration in Chelonia, McCutcheon (1943) stressed the rôle of the flank cavity muscles and showed that throat movements played no significant part in respiration. In our previous work (1954_a) we described two flank cavity muscle sheaths, an inner and an outer in Lissemys. The outer muscle sheath consists of the *diaphragmaticus* anteriorly and *transversus abdominis* (Fig. 1) posteriorly, though the boundaries of these two muscles are not clearly demarcated unlike in *Emys europea* (Owen, 1866) or *Malachemys centrata* (McCutcheon 1943). In both Lissemys and Trionyx the outer sheath arises from the inner lateral border of the carapace from the first to the seventh ribs, but in the latter it has a wider area of origin. The fibres then run laterally downwards and then becoming membranous enclosing the viscera laterally and ventrally, gains insertion at a point in the centre of the plastron. In Testudo the whole of the anterior portion (*diaphragmaticus*) of this muscle sheath is membranous, while the posterior part (*transversus abdominis* has the same structure as that of Lissemys. In Geomyda, the *diaphragmaticus* consists of two parts, an anterior one which is fleshy and a posterior one which is membraneous. The posterior membraneous part joins with the fleshy *transversus abdominis* which has a similar disposition to that of Testudo. The nature of the *diaphragmaticus* in Geomyda appears as a transition between that in Lissemys on the one hand and Testudo on the other.

The inner muscle sheath (Fig. 1) present in Lissemys and Geomyda (George and Shah 1954_a, $'55_a$) arises from a small elliptical tract on the dorsum dorsal to the body cavity in the region of the second to the fifth ribs and the fibres run over the lung so as to form not only an envelope around it but even merge with its wall. In Geomyda, however, this muscle consists of fleshy fibres medially, anteriorly and anterolaterally, while in the intervening part it is made-up of a thin membrane. On the contraction of this muscle sheath, expiration is brought about and on relaxation inspiration is effected. Thus the two muscle sheaths enable the lungs to function as a pair of bellows. We also noted that the inner muscular layer occurring on the wall of the lungs is innervated by the intercostal nerves and suggested (1954_a) that it is homologous to the intercostal muscles of other reptiles. The presence of this unique muscle on the lung wall in *Lissemys punctata* and *Geomyda trijuga* encouraged us to look for such a muscle in other Chelonians. Since then we have extended our studies to two more Chelonians, *Trionyx gangeticus* and *Testudo elegans* and found that in these the muscle is absent. It must, however, be mentioned that in place of the fleshy muscle bag found in Lissemys a thin membrane is left on the lung wall of these Chelonians. It has already been mentioned that in Geomyda the muscle bag is not completely fleshy, but has an intervening portion of membrane. So, the condition of the inner muscle sheath found in Geomyda appears to be an intermediate stage between Lissemys on the one hand and Trionyx and Testudo on the other.

The above-mentioned facts lead us to certain important conclusions regarding the evolution of the Chelonia.

(1) On the fusion of the ribs and the formation of the body shell in the evolution of the early Chelonia resulting in the inability of the body wall to perform the respiratory movements, the inner muscle sheath came to be formed probably from the intercostal muscles. This acquisition of a muscular lung working as a pair of bellows must have given the early Chelonians their survival value.

(2) The study of the flank cavity muscles reveals a general degeneration of some of these muscles in Testudo and Trionyx. In both these forms the inner muscle sheath which is so well preserved in Lissemys is reduced to a mere membrane and the condition in Geomyda shows a stage of transition. Again, in Geomyda the *diaphragmaticus* part of the outer muscle sheath, shows a transitional stage between the one in Lissemys and Trionyx on the one hand and Testudo on the other.

(3) In the nature of the flank cavity muscles therefore, Lissemys and Geomyda are to be regarded as more primitive than Trionyx and Testudo with Geomyda as an intermediate form between Lissemys and the other two. Some evidence in support of this is available from the recent work of Williams *et al.* (1952) who on the basis of their studies on the plastron in *Testudinata* and *Trionychidæ* have suggested that *Lissemydinæ* are a primitive group.

(4) The probable cause for the loss of the inner muscle sheath of the lung of the higher Chelonia and the degeneration of the *diaphragmaticus* in the terrestrial forms like Testudo could be the evolution in these animals of larger lungs on which the muscle bag was more of a hindrance to their full expansion ; and in the mean-time the aquisition of a better physiological equipment for fixing a greater amount of oxygen store in the body for gradual and slow utilization. A physiological study of the lung and blood of these animals is therefore undertaken and is in progress.

The *obliquus externus* (Fig. 1) in Lissemys arises as a flat fleshy belly from the lateral margin of the carapace at the level of the first and second marginals and the fibres run obliquely, medially and posteriorly and becoming tendinous join with the tendinous part of the outer muscle sheath (*transversus abdominis* part) of the lung and closely enveloping the visceral organs to gain a common insertion on the pubic symphysis and on the hypoplastron. In Trionyx also the *obliquus externus* has the same origin, course and insertion as in Lissemys. In Lissemys, on the ventral side of the *obliquus externus* and *obliquus internus* (Fig. 1) there is a ligamentous tissue which arises from the hypoplastron and the first marginal and gets inserted on the fascia of the gluteal and the *rectus femoris* muscles. A part also adheres very closely to the skin covering the shank. This ligament perhaps is useful to retract the leg under the femoral valves, of the plastron. In Lissemys and Trionyx at the junction of the tendinous parts of the *obliquus externus* and *obliquus internus* a small flat muscle which runs downwards and gets inserted on the outer border of the pubic spine takes its origin. This is perhaps the continuation of the *obliquus externus* and *obliquus internus* muscles.

The *obliquus internus* in Lissemys has its origin, medial to that of the *obliquus externus*, namely on the outer border of the costal plates corresponding to the third to the fifth marginals. The fibres run obliquely forwards and inwards and finally become tendinous and get inserted on the dorsal part of the pubic symphysis. In Trionyx too the *obliquus internus* has the same disposition.

In Testudo and Geomyda, however, instead of two separate muscles there is a single muscle, the *obliquus externus*, bordering the lateral side of the posterior half of the carapace. This single flat muscle in these Chelonians is similar to the one found in *Emys europea* described by Owen (1866). The muscle fibres run obliquely forwards and inwards which finally get inserted on the hypoplastron and xiphiplastron. In Testudo and Geomyda therefore the *obliquus internus* is either absent or is merged with the *obliquus externus.*

The *retrachens capitis collique* (Fig. 2) in Testudo arises on the entire side of the vertebral column on the ventral side by four fleshy fasciculi, from the neural plates covering the third to the seventh trunk vertebræ. Of the four, the posterior one is very massive and arises from the neural plate covering the sixth trunk vertebra and also from the anterior half of the neural plate covering the

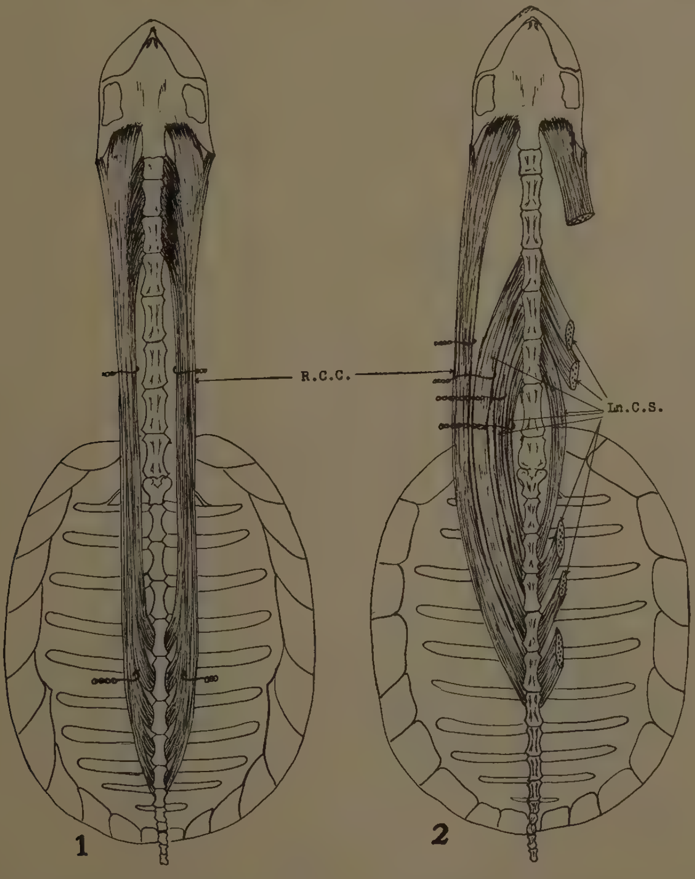

Fig. 2. 1. Geomyda ; 2. Testudo.
L.M. C.S., muscles of the longus colli series in thetrunk; *R.C.S.*, retrachens capitis colligue.

seventh vertebra. The muscle runs anteriorly and enters the neck and after traversing through the entire length of the neck almost all the fibres get inserted by a thick tendon on the basioccipital on the ventral side of the skull, while a few gain separate insertion on the inner posterior border of the squamosal by a thick tendon. The two fasciculi next to the one described above arise from the side of the fifth and fourth trunk vertebræ respectively and both run side by side anteriorly in order to enter the neck region. The one which arises from the side of the fifth trunk vertebra and its corresponding neural plate gets inserted on the diapophysis of the fourth cervical vertebra and the one which

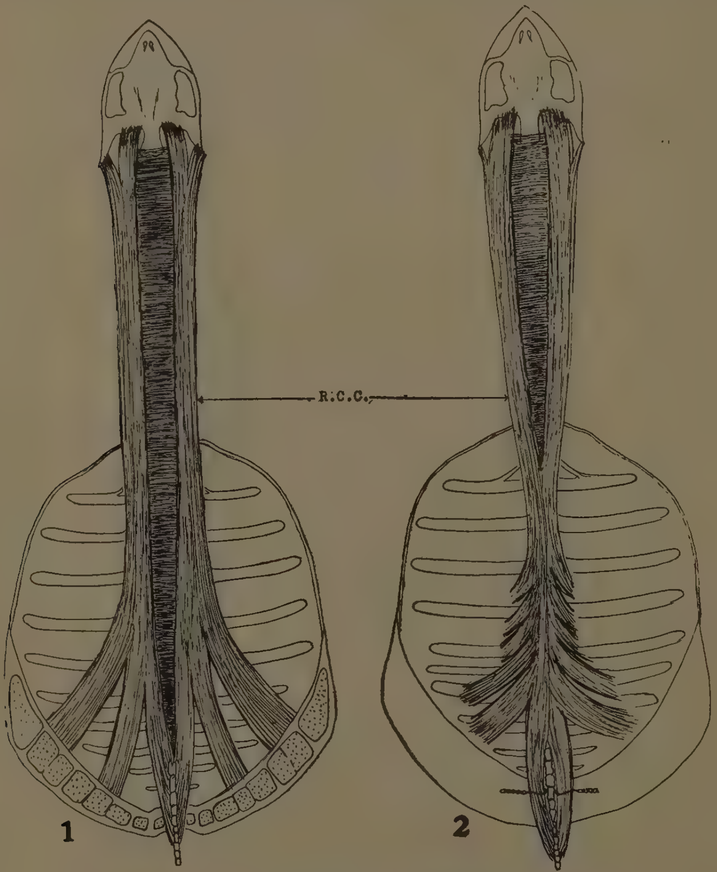

FIG. 3. Ventral view showing the Retrachens Capitis Collique Muscle: 1. Lissemys: 2. Trionyz.

R.C.C., retranchens capitis collique.

arises from the side of the fourth trunk vertebra and its corresponding neural plate gets inserted on the diapophysis of the fifth cervical vertebra. The anterior-most fasciculus arises from the third trunk vertebra and its corresponding neural plate, runs anteriorly and after entering the neck gets inserted on the diapophysis of the sixth cervical vertebra. In describing the *retrachens capitis collique* in *Emys europea*, Owen (1866) states that it arises by six fleshy fasciculi from the neural arches and spines of the dorsals and proceeds forwards blending together and gets detached into four tendinous insertions. But in Testudo there are only four such fasciculi and they remain completely separate without any blending whatsoever. On studying the nerve supply to these four fasciculi it is found that the posterior-most one, the largest and longest fasciculus, which is inserted on the base of the skull is innervated by a branch from the middle cervical ganglion of the vagus and the sixth thoracic nerve at the region of its origin, whereas the others, the anterior three fasciculi, obtain their nerve supply from the third, fourth and fifth thoracic nerves. The fact that these anterior three fasciculi remain completely separate from the posterior large fasciculus and that they are innervated by a totally different source of nerve supply tends to show that they are different muscles altogether. It is suggested that they correspond to the *longus colli* series of muscle of the trunk and that the posterior large belly is a composite muscle formed anteriorly by the *sterno-cleido-mastoid* as was suggested by us in the case of Lissemys (1954_a) and posteriorly by the last fasciculus of the *longus colli* series (George and Shah 1954_b).

The *retrachens capitis collique* in Lissemys (George and Shah, 1954_a) is a highly specialized muscle and considerably different from that of the Testudo in its attachments. In Lissemys it arises by three fleshy parts. The lateral one arises from the junction of the first and second marginals and their corresponding costal plates, the middle one arises close to the lateral one from a point posterior to its place of origin, while the medial part arises from the last costal plate at the posterior region near the vertebral column and from the bodies of the five anterior caudal vertebræ. All these parts on each side run anteriorly for a short distance when the lateral and middle ones join with the medial one to form a single massive muscle belly on either side and proceeds towards the head. This linear muscle column on each side runs through the entire length of the region of the trunk and neck to gain a tendinous insertion on the basioccipital and partly on the posterior inner border of the squamosal of the respective sides. These bellies on either side are connected to one another throughout their course from the place of origin to that of their insertion by a thin aponeurosis, which is narrower in the posterior region and gradually becoming broader as the muscle proceeds anteriorly separating the two bellies more and more laterally. On approaching the base of skull each divides into two, a lateral and a medial part. The former part which is comparatively a minor one gets inserted on the inner posterior border of the squamosal and the latter on the basioccipital and the posterior part of the pterygoid, both the insertions being tendinous. It has been suggested by us (1954_a) that the *retrachens capitis collique* muscle in Lissemys is divisible, arbitrarily, into two parts at the level of the episternal plate (epiplastron) of the plastron. These two parts of the muscle have a different nerve supply; the anterior, cervical part being innervated by a branch from the middle cervical ganglion of the vagus, while another branch from the same ganglion innervates the *rectus capitis cervico-plastratis.* From this we had ventured to homologise, the anterior, cervical part of the *retrachens capitis collique* with the *sterno-cleido-mastoid* muscle which is derived from the *rectus capitis cervico-plastralis*, the primitive *cucularis.* The posterior trunk part has two sources of nerve supply: anteriorly by the fifth cervical nerve and posteriorly by a nerve from the lumbo-sacral plexus and the twelfth dorsal. We had suggested (1954_a) that owing to the skeletal modifications in the formation of the carapace and plastron, the *sterno-cleido-mastoid* muscle did not get its usual attachment but joined with the posterior part which is in turn derived from the *pubo-ischio-femoralis*, from which the *psoas* muscles of mammals were derived. The twelfth dorsal nerve which supplies the posterior-most part of the *retrachens capitis collique* innervates the *pubo-ischio-femoralis* muscle in addition.

In Trionyx it consists of six fasciculi of which the anterior first, second and third are very thin and membranous, while the fourth and fifth are massive and well-developed. The last fasciculus which is also a well-developed one, arises from the lateral surfaces of the body of the second, third, fourth and fifth caudal vertebræ. The fourth and fifth fasciculi arise from almost the entire ventral surface of the costal plates covering the ribs between the seventh and eighth, and the eighth and ninth trunk vertebræ. The first, second and third fasciculi arise from the neural plates corresponding to the fourth, fifth and sixth trunk vertebræ respectively. The first two are very thin, while the third one is somewhat fleshy and well-developed. All these fasciculi of one side blend together to form a single muscle belly which runs anteriorly through the trunk and neck. At the level of the eighth or ninth trunk vertebra the muscles of either side fuse with the one another to form a single stout muscle mass. Such a blending of the muscle of either side is completely absent in Testudo and Geomyda, but in Lissemys though they do not fuse with one another directly, there is an indirect connection through an aponeurosis which is very narrow at the posterior end. In Trionyx after the fusion, forming a single muscle, it runs anteriorly forwards into the trunk region, but at the level of the first trunk or the last cervical vertebra, the component parts part company at the same time retaining their connection with each other by a thin aponeurosis which is narrower in this region, and later widens out separating the muscles more laterally, a condition very much similar to the one observed in Lissemys. After reaching the base of the skull the muscle splits up into two, a lateral and a medial part. The latter which is the major part gets inserted on the basioccipital and the posterior part of the pterygoid by a thin tendon, while the former gets inserted on the 'posterior inner border of the squamosal. In this animal the insertions of this muscle are similar to those of the same muscle in Testudo, Geomyda and Lissemys. Since the muscle in Trionyx consists of six fasciculi of which the last three arise from very much similar places of origin as in Lissemys, this may be regarded as homologous to the same part of the muscle in Lissemys, whereas the anterior part comprising the first three fasciculi in Trionyx are similar to those seen in Testudo and Geomyda, but at the same time it differs in that that all the fasciculi in Testudo do not blend together to form a single muscle as in Trionyx. In this respect of blending, Geomyda differs from Testudo in having the blending of all the fasciculi to form a single muscle belly, almost similar to the one in Trionyx and Lissemys, such a type of blending of the different fasciculi is present in *Emys europea* (Owen 1866). The muscle in Trionyx after the blending remains as a single mass and runs forwards in the trunk and through the neck to gain its insertion similar to the one found in Lissemys. So in Trionyx it is suggested that the first three fasciculi of this muscle are similar to those of Testudo which we have suggested above to be homologous to the *Longus colli* series of muscles.

In Geomyda this muscle arises by five fleshy fasciculi from the body of the fifth, sixth, seventh, eighth and ninth trunk vertebra, and the neural plates covering these vertebræ. All the fasciculi of one side blend together and form a single muscle which runs anteriorly through the trunk and enters the neck. After reaching the third cervical vertebra, some median fibres become tendinous and get inserted on the lateral side of the bodies of the first, second and third cervical vertebræ, while the major part of the muscle runs forwards and gets inserted on the basioccipital by a tendinous insertion and a few lateral fibres also become tendinous to get inserted on the inner posterior border of the squamosal. The retrachens of either side, though runs side by side, does not show any fusion or connection with each other by aponeurosis. The posterior part of this muscle consisting of the five fasciculi which have blended together corresponds to the *longus colli* series, while the anterior part to the *sterno-cleido-mastoid* muscle. It must, however, be mentioned that the insertion on the bodies of the first three cervical vertebræ of the median fibres of the anterior part which is absent in Testudo, indicates that these fibres are of the *longus colli* series and that if this inference is true, the anterior part is formed by both *sterno-cleido-mastoid* (mainly) and the *longus colli* series.

This composite muscle in its reduction in Trionyx and Geomyda shows a condition somewhat intermediate between that of Lissemys on the one hand and Testudo on the other in which the reduction is maximum. It must be mentioned that of the two, Trionyx is nearer to Lissemys, and Geomyda to Testudo.

Muscles of the Tail. (Fig. 4).

In the Chelonia the tail has lost its importance as it is neither an organ of locomotion nor of defence and offence, as in the other reptiles and presents no direct muscular link up with the thigh so as to move with the movements of the posterior extremity. This has therefore brought about the great reduction of the muscles in this region of the body.

In Lissemys and Trionyx the tail is extremely small. Compared to this the tail in Geomyda or Testudo is considerably longer and extends beyond the cloacal opening. In all these chelonians as in other reptiles there is a superficial muscle sheath, the *flexor caudæ superficialis*, covering the deep caudal muscles on the ventral and lateral sides of the tail. The deep caudal muscles of Chelonia, the *flexor caudæ profundus* in general do not show their normal attachments. The median dorsal muscles comprising the *spinalis-semispinalis* system of muscles show the typical criss-cross arrangement that is so characteristic of reptils. This arrangement assuring free intervertebral movements is retained only in the tail region of the chelonian, even though the tail is very much reduced and no longer functions as an active organ. So it becomes evident that the criss-cross type of arrangement in the *spinalis-semispinalis* system that is so typical of all modern reptiles, must have been present not only in the immediate ancestor of the Chelonia but also in the early reptilian ancestors as well, from which all the modern reptilian forms were evolved.

The *flexor caudæ superficialis* muscle is present as a superficial muscle sheath covering the sides of the cloaca and the deeper muscles of the tail. It arises from the neural spines of the caudal vertebræ and also from the medial part of the last costal plate. The fibres run laterally and downwards and join with those of the opposite side forming a thin aponeurosis on the mid-ventral line of the tail. Proximally the aponeurosis gets attached along the dorsal side of the ischial symphysis, while distally the muscle remains fleshy and free from any attachments. The posterior-most part of this muscle is more muscular and becomes the sphincter of the cloaca. The *flexor caudæ superficialis* is homologised by Haines (1935) to the *ilio-coudalis* and *ischio-caudalis* of lizards and crocodiles. In Lissemys, Trionyx and Geomyda, however, this muscle seems to correspond to the *ilio-caudalis* only, though has lost its normal attachment to the ilium. The *ischio-caudalis* is a well-developed muscle in these three chelonians. In *Testudo pardalis*, Haines (1935) mentions an unidentified muscle arising from the lateral side of the body and transverse processes of the caudal vertebræ and inserted on the pubis. We too have come across a similar muscle in *Testudo elegans* but not in Lissemys, Trionyx and Geomyda. This leads us to the inference that the unidentified muscle in *Testudo pardalis* and *Testudo elegans* should correspond to the *ischio-caudalis* of Lissemys, Trionyx and Geomyda, since there is no such muscle arising from a caudal vertebra and getting inserted on the pubis in Lissemys, Trionyx and Geomyda, though a few fibres of this muscle in Geomyda do get inserted on the pubis. The manner of the insertion of this muscle in Geomyda denotes a condition intermediate between that in Lissemys and Trionyx on the one hand and Testudo on the other. That in Testudo this muscle instead of having its insertion on the ischium, gets itself attached on the dorsal region of the pubic symphysis and that this shift had occurred in this animal could perhaps be visualised if it is realised that the relative positions of the ischium and pubis in Testudo are at a greater angle than that is seen in Lissemys or Trionyx in which they are more or less in the same line or at least at a relatively small angle. The position of these bones in Geomyda is somewhat at a greater angle than that in Lissemys and Trionyx but slightly lesser than that in Testudo. It must also be mentioned that the ischium and pubis in Testudo and Geomyda are placed very close to each other separated by a very small ischial foramen, whereas in Lissemys and Trionyx, they have a considerable distance between them being separated by a large ischial foramen. From this arrangement of the two bones in Testudo and Geomyda, it is possible to visualise a change having taken place in the point of insertion of the unidentified muscle of Haines (1935) in Testudo, from the ischium to the pubis, and we therefore venture to call this muscle, *ischio-caudalis* and consequently regard the *flexor caudæ superficialis* as consisting of the *ilio-caudulis* only and not as a composite muscle formed by the *ilio-caudalis* and *ischio-caudalis.*

The sphincter part of the *flexor caudæ superficialis* which aids the opening and the closing of the cloacal aperture, is retained in the mammals as the perineal muscles, while the other part is somewhat degenerated (Haines, 35). Laterally on the tail in Lissemys just by the lateral border of the *flexor caudæ superficialis* there is a small muscle on both the sides which arises from the anterior border of the last marginals and gets inserted on the caudal valve of the plastron. This muscle is referred to by Hassan (1941) as the *margino-infra-caudalis.* It is very likely that it is only a part of the *flexor caudæ superficialis* and the nerve supply to both these is derived from the same source. This muscle brings about the closure of the caudal valves of the carapace and plastron after the retraction of the tail into the shell. Such a muscle is totally absent in Trionyx, Testudo and Geomyda since such caudal valves are absent in these animals.

The *flexor caudæ profundus* of Chelonia is homologised with the *caudo-femoralis* of lizards (Haines, 1935). Since the tail has been reduced and has lost its function as an organ of locomotion, the attachment of the muscle on the femur is shifted to the costal plate near the vertebral column at the level of the sacrum. In Lissemys it arises lateral to the origin of the *ischio-caudalis* from eight caudal vertebræ and runs anteriorly forwards to gain insertion on the last costal plate just near the sacral vertebræ. A few fibres are also inserted on the lateral side of the body of the sacral vertebræ. In Trionyx the origin is almost similar to that in Lissemys but does not cover such an extensive area of origin where it arises from only four caudal vertebræ starting from the eleventh caudal. The muscle runs anteriorly and gains insertion on the costal plates near the

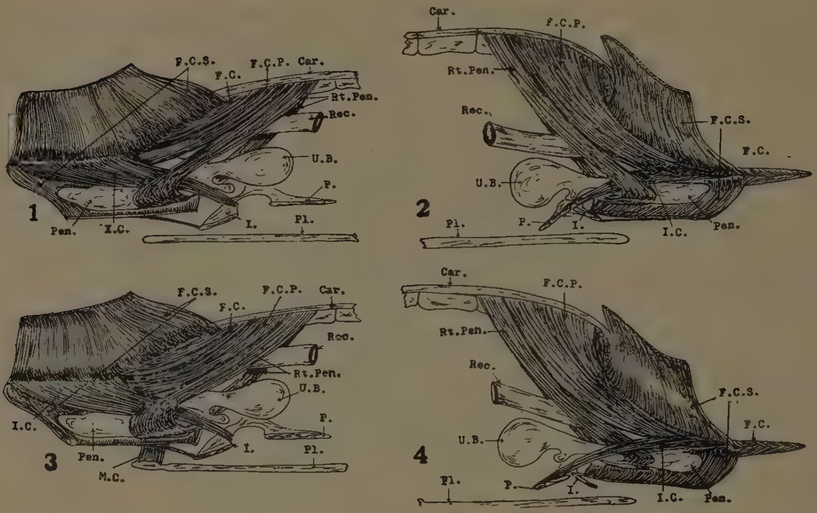

FIG. 4. Lateral view showing the Muscles of the Tail : 1. Lissemys ; 2. Testudo ; 3. Trionyx 4. Geomyda.

Car., carapace ; *F.C.*, flexor caudæ : *F.C.P.*, flexor caudae profundus ; *F.C.S.*, flexor caudæ superficialis ; *I.*, ischium ; *I.C.*, ischio-caudalis ; *M.C.*, margino-infra-caudal muscle ; *P.*, publis ; *Pen.*, penis ; *Pl.*, plastron ; *Rec.*, rectum ; *Rrt. Pen.*, retractor penis ; *U.B.*, urinary bladder.

first sacral vertebra. A few fibres gain insertion also on the body of the vertebra as seen in Lissemys. In Testudo it is well-developed and arises from the last six caudal vertebræ and gets inserted on a very extensive area on the costal plate corresponding to the eighth and ninth trunk vertebræ. In all these chelonians it acts as the flexor of the tail. The shift of insertion of the muscle from the femur to the costal plate and the muscle becoming the *flexor caudæ profundus* instead of remaining as *caudo-femoralis* as in lizards and crocodiles are accountable since the tail in the chelonians has lost its function as an active organ of locomotion or defense and offense as in the case of lizards and crocodiles. In lizards and crocodiles the tail bends on one side as the leg of the same side moves forwards. This action of the tail in relation to the leg movements helps in propelling the body ahead. A movement of this kind is possible only if the tail is a massive one and it touches the ground. The tail in Chelonia in general is very much reduced and does not touch the ground or even if it does it is so ill-developed and is of so little consequence that the propulsion of the body, which has become very bulky, unwieldy and heavy, becomes impossible. In forms which swim, the question of the tail touching the ground and the propulsion of the body by its action does not arise at all and so the tail is much reduced and ill-developed in them too. Such a change has taken place in the nammals also. (Haines, 1935).

The *retractor penis* is very well-developed in all male chelonians, while the corresponding one in the female is ill-developed and is attached to the clitoris. It arises from the last costal plate closely adjacent to the sacral vertebræ. The fibres run posteriorly downwards and turn over to the ventral side of the cloaca to be inserted on the wall of the cloaca where the penis or clitoris is situated. The muscle during its course runs over the *ischio-caudalis* and at the point of insertion the fibres of the muscle of either side run side by side and gain their insertion. This muscle acts as its name indicates as the retractor of the penis or clitoris.

Besides these long caudal muscles, there are the short muscles on the dorsal and the ventral sides of the caudal vertebræ. The dorsal group in acting as the extensors and the ventral ones as the flexors of the tail vertebræ bring about intervertebral movements.

On the dorsal side the extensor muscles consist of the *spinalis* and *semi-spinalis* (*extensor caudæ*, of Owen 1866) muscles showing the primitive segmental and the typical reptilian (criss-cross) arrangements. *A spinalis* muscle arises from the posterior border of the transverse process of one vertebra and gets inserted on the region of the neural spine of the next anterior vertebra. A *semi-spinalis* arises from the base of the transverse process and also from the side of the neural arch of one vertebra and running forwards and outwards gains insertion on the posterior border of the transverse process of the next preceding vertebra. In all the chelonians studied, the *spinalis-semispinalis* system of the first three caudal vertebræ are very well-developed and massive. They are inserted on the posterior border of the last costal plate by the side of the vertebral column. Under cover of the *semi-spinalis* are the short vertebral muscles, the *intertransversarii* which extend between two transverse processes.

On the ventral side of the tail are the flexor muscles called *flexor caudæ inferior* (Owen 1866) arising from the transverse process of one vertebra and inserted on the side of the body of the next preceding vertebra. The muscles arising from the first three caudal vertebræ are well-developed and are inserted on the body of the second sacral vertebra and also on the costal plate covering the sacral vertebræ. This set of caudal flexors present in all the chelonians we have examined, is called *flexor caudæ lumbalis* by Owen (1866).

Summary

The muscles of the trunk and tail, in the four common chelonians namely *Lissemys punctata*, *Trionyx gangeticus*, *Geomyda trijuga* and *Testudo elegans* have been described and the following are some of the salient findings.

1. The carapace in Testudo and to a lesser extent in Geomyda having acquired the shape of a dome and the neck having undergone an unextendable

bend, unlike in Lissemys and Trionyx, a great reduction in the *spinalis-semispinalis* system of muscles inevitably resulted.

2. In the evolution of the Chelonia, the lung acquired a striated muscle bag probably derived from the *intercostal* muscles, which is retained in Lissemys and to a lesser extent in Geomyda, but is reduced to a mere membrane in the others.

3. The *diaphragmaticus* muscle which is well-developed in Lissemys and Trionyx is reduced to a mere membrane in Testudo and to a slightly lesser extent in Geomyda.

4. The *retrachens capitis collique* is a highly modified and composite muscle in Chelonia and it is suggested that it is formed by the *sterno-cleido-mastoid* anteriorly and the *longus colli* series of the trunk and the *pubo-ischio-femoralis* posteriorly in Lissemys, while in the others by the *sterno-cleido-mastoid* and the *longus colli* series only.

5. The *flexor caudæ superficialis* of the tail, it is suggested, corresponds to the *ilio-caudalis* and that the hitherto unidentified tail muscle in Testudo to the *ischio-caudalis* of other reptiles.

6. From the present study it is evident that, of the four Chelonians studied, Lissemys is the most primitive and Testudo the most modified, with Geomyda occupying an intermediate position, while Trionyx is closer to Lissemys than to the others.

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THE TRUNK MUSCLES OF *PANULIRUS POLYPHAGUS* (HERBST)

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The chief muscles of the Crustacea are the well-developed longitudinal ones (axial muscles) which in Panulirus and others are concerned with the straightening, bending and other movements of the abdomen. There are also the muscles of the appendages, the appendicular muscles which govern their movements.

The axial muscles in Panulirus can be classified into the extensor muscles which bring about a straightening of the abdomen and the flexor muscles which act in such a way as to bend the abdomen inwards to the ventral side.

The extensor muscles comprise, the dorsal extensors, the extensors of the telson and the inter-tergal abdominal muscles. The flexors consist of the major flexors, the accessory flexors, the ventral thoracico-abdominis, the lateral thoracico-abdominis, the flexors of the telson and the ventral superficial flexors.

The Extensors

The dorsal extensors: These consist of two pairs of longitudinal bands lying on either side of the mid-dorsal line in the abdomen. Of these the inner pair is thicker than the outer. The cross section of the inner muscles is an isosceles triangle, the broad base of which rests on the flexor muscles which lie below. These muscles are divided into broad sheets of bundles (fasciculi) which wind round one another like the shreds of a twisted cord. These windings and the consequent twisted arrangements of the bundles are clear in the cross section.

The twisted disposition of the muscle bands makes the lines of force converge to a point which lies along the axis of the muscle block. Such an arrangement does not interfere with the sideway deflections of the segment. On the other hand the twisted bands are longer and stronger by far than they would have been if they were straight and of one piece.

The muscle bands of the outer pair of the dorsal extensors are not wound round one another as in the inner pair, though their attachments are to the same intersegmental membranes. In their action therefore there is no possibility of a lateral deflection, but only a linear drawing in of the posterior segments to the anterior.

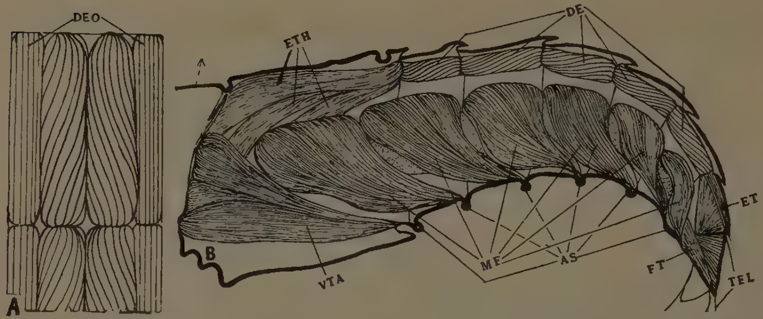

Fig. 1. *A.*—A dorsal view showing the two pairs of Dorsal Extensor muscles of a segment.
B.—A dissection of Panulirus from the left side showing the Dorsal Extensors and the Major Flexors of the trunk.
AS, abdominal sterna ; *DE*, dorsal extensors, inner pair ;
DEO, dorsal extensors outer pair; *ETH*, extensors, of the thorax; *ET*, extensor of telson ; *FT*, flexor of telson *MF*, major flexors ; *TEL*, telson ; *VTA*, ventral thoracico-abdominis.

Though the dorsal extensor muscles appear as continuous longitudinal bands, they are segmental muscles. Each division of it (myomere) runs from segment to segment, though in action all the parts can contract simultaneously and produce the cumulative effect of straightening the whole abdomen. Their attachments in Panulirus are not to the terga proper as described by T. Huxley in Astacus or by Patwardhan in Palæmon, but by connective tissue strands to the intucking intersegmental membranes.

In the thorax, the segmentation of the extensor muscles is lost and they occur in five muscle bands on either side, which as we observe from the abdominal end spread fan-wise and are seen attached to the lateral sides of the carapace. This attachment in fact constitutes the origin of the dorsal extensors. The outer pair of the dorsal extensors seems to contribute wholly to the uppermost pair of the five bundles and also to the lower pair which lies next. The others are continuous wholly from the inner pair.

Of the five bundles mentioned above, the topmost is the longest, and the lowest the shortest, with progressively decreasing lengths between.

As indicated before the dorsal extensors by their contractions draw the anterior edge of each tergum under the posterior edge of the preceding segment. The total effect of such contractions is to straighten the abdomen. To a limited extent alternate contractions of muscles of either side produce lateral deflection of the segments.

The extensors of the telson : The extensors of telson are two pairs. One pair consists of the dorsal extensors which are represented in the sixth abdominal segment by a single pair of muscles. They are slender muscle bands attached at one end to the membrane between segments 5 and 6 and at the other end to small calcified chitinous plates developed on the intersegmental membrane between the sixth abdominal segment and the telson. The second pair is short and thick arising from the sixth abdominal sternum and attached to the entire front margin of the roof of the telson. Contraction of these two pairs brings about a straightening of the telson.

The inter-tergal abdominal muscles : These are five pairs of short longitudinal bundles. Each pair is made up of dorso-lateral portions which connect the dorso-lateral walls of the two adjacent abdominal terga and a transverse portion which joins the dorso-lateral portions with one another in the same segment. The transverse part is the remnant of the ancestral circular muscles. The dorso-lateral portion of every pair arises from the dorso-lateral wall of the tergum of one segment and extends backwards to be inserted on the dorso-lateral wall of the tergum of the following segment above the level of the hinge joint. It is slightly concave above and convex below and has the appearance of a loop. They connect the segments 1 and 2, 2 and 3, 3 and 4, 4 and 5, and 5 and 6.

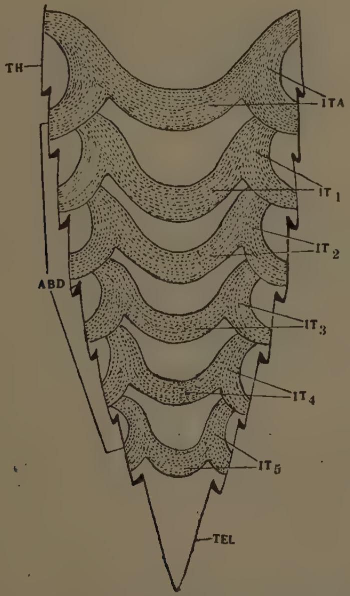

Fig. 2. A diagramatic view of the Inter-tergal muscles of abdomen and thorax.
ABD, abdomen ; *ITA*, inter-tergal muscle between thorax and abdomen; *IT* 1—5, inter-tergal abdominal muscles ; *TEL*, telson; *TH*, thorax.

In Palæmon only four pairs of inter-tergal abdominal muscles are present, there being no representatives between the fifth and sixth abdominal segments. The inter-tergal muscles are situated above the level of the hinge joints. By the contraction of the transverse portions of these muscles, the dorso-lateral portions of each tergum are pulled towards one another. Along with this the dorso-lateral portions also contract so as to telescope the terga of a segment into that of the preceding one. The inter-tergal muscles thus supplement the action of the dorsal extensors.

The inter-tergal muscles between the abdomen and the thorax: These are a single pair of longitudinal muscles one on either side connecting the thorax with the first abdominal segment. Each of these muscles arises from the lateral wall of the anterior part of the thorax as three muscle bands. But they very soon unite together and run backwards as a single muscle for a short distance and again resolve into three components of which the lowest is very slender and runs as far as the first abdominal sternum. The two upper bands run backwards and again unite. Part of this band which is formed as a result of the union of the two upper strips is inserted on the lateral wall of the first abdominal tergum, while the other short part of it runs inwards and joins with its fellow of the opposite side. The short transverse muscle is homologous with the transverse bands of the abdominal region. At the region where the upper bands mentioned above split and join again, a slit is formed. It may be mentioned here that through this slit, the anterior end of the abdominal flexor emerges inwards and unites with its fellow of the opposite side in the median line.

The contraction of the inter-tergal muscles between the thorax and the abdomen pulls the first abdominal tergum forwards under the posterior margin of the carapace and thus helps in the extension of the abdomen. There is no doubt that in function and position these muscles correspond to the inter-tergal abdominal muscles.

That the lower most band of this inter-tergal muscle which connects the thorax with the abdomen is attached below the level of the hinge joint in the abdominal segment.

These muscles like the inter-tergal muscles of the abdomen bring telescoping of the first abdominal segment slightly into the thoracic portion.

The Flexors

The major flexors: These are six pairs of strong muscles, by far better developed than the extensors. Their development is in correlation with the sudden and quick flexsions of the abdomen which bring about rapid backward movements which are characteristic of the animal. The fasciculi of these muscles are twisted like the strands of a rope as in the inner pair of the extensors. This adds to their cumulative strength. The six pairs of major flexors correspond to the six abdominal segments. In Palaemon the major flexors are described as five pairs of muscles there being no flexors in the sixth abdominal segment. The flexor muscles which arise from the sternum of the second abdominal segment may be taken as a type. Each of this pair takes its origin from the second abdominal sternum near the mid-ventral line, proceeds obliquely backwards and upwards and after looping round the inter-tergal abdominal muscle between the third and fourth segments comes to lie on the inner side of the inter-tergal muscles mentioned above. It then runs obliquely downwards and backwards becomes inserted on the sternum of the fifth abdominal segment towards the side. It may also be said that a major flexor though it arises and terminates as a thin strip, broadens and thickens in the intermediate segments through which it passes.

The other flexors which arise from the abdominal segments first, third and fourth segments are disposed as follows:

The pair originating from the first abdominal segment has its anterior attachment to the mid-ventral line of (the chitinous portion of) the sternum of that segment. Each extends to segments 2 and 3 and terminates on the sternum of the fourth segment in the mid-ventral line.

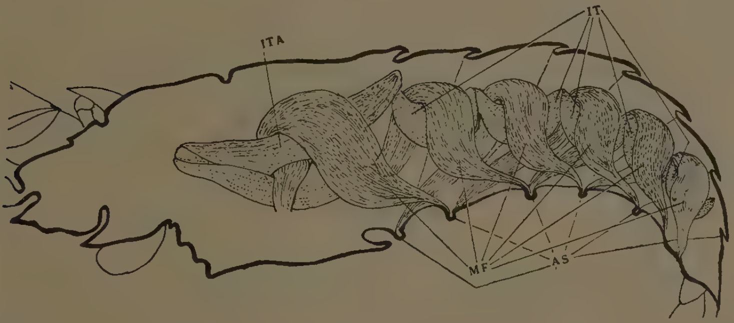

Fig. 3. A disssection of Panulirus from the left side showing the Major Flexors and the Inter-tergal muscles of the trunk.
AS, abdominal sterna; *ITA*, inter-tergal muscle between thorax and abdomen, *IT*, inter-tergal muscles, *MF*, major flexors.

The major flexors arising from the third abdominal sternum proceed to segments 4 and 5 and terminate on the anterior margin of the sixth abdominal sternum.

The major pair of flexors originating from the fourth abdominal sternum proceeds to segments 5 and 6 and terminates on the posterior margin of the sternum of the sixth abdominal segment.

In addition to these, two pairs of well-developed major flexors are present, one pair in the anterior region of the abdomen and the other in the posterior region of the thorax. Of these the two flexors which make up the anterior pair are fused with one another at their origin which lies at the ventral portion of the thoracic cavity. As already mentioned, the two components pass through the slits (on either side) left between the two upper bands of the inter-tergal muscles between the thorax and first abdominal segment. Finally they terminate on the second abdominal sternum. The posterior pair arises from either side of the mid-ventral line of the first abdominal sternum. This point of origin is very near to the termination of the inter-tergal muscles between the abdomen and the thorax. Their termination is on the third abdominal sternum.

In Palæmon, the major flexors which arise from the fourth abdominal sternum and terminate on the posterior margin of the sixth abdominal sternum are absent.

It is also noteworthy that in Palæmon the flexors which correspond to the anterior-most pair described here are attached to the lateral wall of the thorax and are not united with one another as in Panulirus.

The major flexors by their contraction pull the successive sterna closer together and thus bring about the flexion of the abdomen. It should be mentioned here that as the flexor muscles loop over the inter-tergal muscles, a contraction of the former automatically prevents the contraction of the latter at the same time; the two sets contract and relax alternately and not simultaneously.

The accessory flexors: These are five pairs of muscles found in the abdomen. They are delicate strips very much flattened out. The absence of fasciculi is characteristic of these. The first pair arises from the sternum of the second segment one on either side of the origin of the major flexor. They then extend obliquely forwards and upwards between the inter-tergal muscles which connect the first and second abdominal segment posteriorly. Only about a third of their length lies in the first abdominal segment. They are inserted on the tergum of the first abdominal segment above the level of the hinge joint between these segments. The accessory flexor muscles of the other segments are similarly disposed and they decrease in length from anterior to posterior segments.

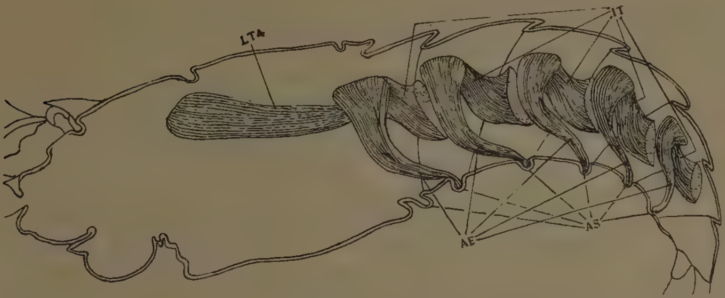

Fig. 4. A dissection of Panulirus from the left side showing the Accessory Flexors and the Inter-tergal muscles of the trunk.

AF, accessory flexors; *AS*, abdominal sterna; *IT*, inter-tergal muscles; *LTA*, lateral thoracico-abdominis.

When these muscles contract, the successive sterna are obliquely pulled forwards and thus bring about a flexion of the abdomen. These therefore supplement the action of the major flexors.

The flexors between the thorax and the first abdominal segment: *The ventral thoracico-abdominis*: These are a pair of very thick bands connecting the thorax with the first abdominal segment. Each muscle band is attached in front to the entire margin of the endophragmal system and partly to the lateral wall of the thorax. It arises from the attachment as several bands which proceed obliquely downwards and backwards and ultimately attach to a pair of calcified plates on the inter-segmental membrane between the thorax and the abdomen. The muscle bands of two sides are situated very close to each other and only a narrow chink is left between them. Thus placed, they form a covering over the endophragmal skeleton with the exception of a chink in the middle line. Through this chink the sternal artery passes through the aperture in the endophragmal skeleton to the sternal sinus. When these muscles contract they draw the sternum of the first abdominal segment forwards and slightly obliquely upwards. In the case of Palæmon the flexors between the thorax and the first abdominal segment are attached in front to the lateral wall of the thorax but not to the anterior margin of the endophragma as in Panulirus.

The lateral thoracico-abdominis: These consist of a pair of longitudinal muscle bands one on either side. In front each of these is attached to the top of the side wall of the thorax immediately below the extensor bands. It proceeds obliquely downwards and backwards and is inserted to the antero-lateral margin of the first abdominal tergum by a tendon. This tendon is situated just in front of the triangular projection of the posterior border of the first abdominal tergum.

Contraction of these muscles pulls the ventral part of the first abdominal segment upwards and forwards in such a way as to bring about a flexion of the first abdominal segment. In the case of Palæmon these muscles occur as two pairs of muscles, a pair on each side and each pair arises from the top of the side wall of the thorax.

The flexor muscles of the telson: These comprise a pair of muscles one on either side. Each muscle is a slender strip which originates from the lateral extremities of the sternum of the fifth abdominal segment and proceeds backwards to be inserted at the base of the telson. Contraction of these muscles flexes the telson.

The ventral superficial flexor muscles: In the sternal region between segments 1 and 2, 2 and 3, 3 and 4, and 4 and 5 are found delicate sheets of longitudinal muscles. A pair of these myomeres are found in each of the inter-sternal region one on either side of the middle line.

Each muscle is short and broad and its fibres remain loose. These muscles do not connect the adjacent sterna as in Palæmon, but each muscle arises from the inter-sternal membrane about mid-way and is attached to the sternum behind. The ventral superficial muscle bands flexes the inter-segmental membrane and thus help the other flexor muscles in their actions.

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THE PERICARDIAL MEMBRANE AND ITS ROLE IN CRUSTACEAN CIRCULATION

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ALEXANDROWICZ (1932) in his paper entitled "The Innervation of the heart of the Crustacea" has mentioned, "that all these systems of peripheral neuropile-like structures must be regarded as sensory elements which may have some relation to the respiration and to the circulation of the blood in the body cavities." He has also given diagrams of the pericardial membrane of Astacus and Palinurus showing the nerve supply. This is perhaps the only reference regarding the possibility of the pericardial membrane having a part to play in the circulation of Crustacea.

While working out the anatomy of *Panulirus polyphagus* we have found that the pericardial membrane is attached anteriorly to the carapace, posteriorly to the inter-segmental membrane between thorax and abdomen, and laterally to the epimeral wall. It is pierced by the outgoing arteries, as well as by the muscles, but all these attachments are fluid tight without allowing the blood to pass out directly from the sinus. That into the pericardial sinus open the brachio-pericardial channels through which the blood flows from the sterno-visceral sinus towards it has been established by T. H. Huxley and subsequent workers in Astacus and other crustaceans.

The following investigations have been conducted to elucidate the structure and nerve supply of the pericardial membrane and also to find out its rôle in the mechanism of circulation.

Material and Methods

Structure of the pericardial membrane :—Several specimens of *Panulirus polyphagus* have been dissected out to find out the disposition, relations, and nerve supply to the pericardial membrane. Some membranes have been fixed stained and mounted.

Action of the pericardial membrane :—After exposing from the dorsal side the heart, and the pericardial membrane from the ventral side, the movements of the heart as well as those of the membrane were noted. Then the heart was connected to a lever attachment recording on a revolving drum. A pericardial nerve was then stimulated electrically by fine electrodes connected to the secondary of a du Bois induction coil the primary of which was excited by a two-volt battery.

Tracings of the heart movements with and without stimulation of the peircardial nerve were obtained.

The tracings of the up and down movements of pericardial membrane were also made by connecting the membrane alone to the lever attachment.

Results.

The pericardial membrane which is tendinious in nature is found arched up, in the normal position. Fig. 1, is a diagrammatic view of the pericardial membrane, its attachment and nerve supply as seen from the ventral side. There are five transverse pairs of striated muscle bands investing the ventral surface of the membrane and directed laterally along it.

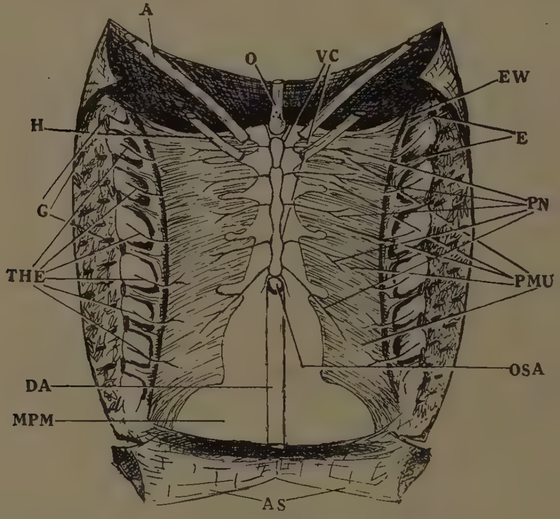

Fig. 1. A ventral view of the pericardial membrane of Panulirus.

A, antennary artery; *AS*, first abdominal segment; *DA*, dorsal aorta; *E*, endophragma; *EW*, epimeral wall; *G*, gills; *H*, hepatic artery; *MPM*, membranous portion of pericardial membrane; *O*, ophthalmic artery; *OSA*, opening of the sternal artery; *PMU*, muscles of the pericardial membrane; *PN*, pericardial nerves; *THE*, thoracic extensor nerves; *VC*, ventral cord.

Excepting the posterior pair all the muscle bands are attached to the epimeral wall; the posterior pair is for the most part attached to the epimeral wall with a small segment extending somewhat obliquely backward and attached to the intersegmental membrane between thorax and abdomen. The nerve supply of the pericardial membrane consists of a pair of longitudinal cords running closely parallel with one another antero-posteriorly along the mid-ventral line of the tendinous part of the membrane. A pair of transverse nerves is given from these cords to each pair of muscle bands. The parallel cords are connected with one another by three transverse connections, one in advance of the first pair of transverse nerves, second in advance of the second pair of nerves and the third behind the last pair. These cords appear to be connected with the thin thoracic extensor segmental nerves issuing from the posterior region of the anterior thoracic ganglion, and also from the posterior thoracic one.

With regards to the movements of the heart and pericardial membrane, the following observations have been made.

1. The pericadial membrane is arched upwards. The depression of the pericardial membrane synchronises with the relaxation of the heart and its elevation with the contraction of the heart.

2. On electrical stimulation of its nerves the pericardial membrane is distinctly depressed.

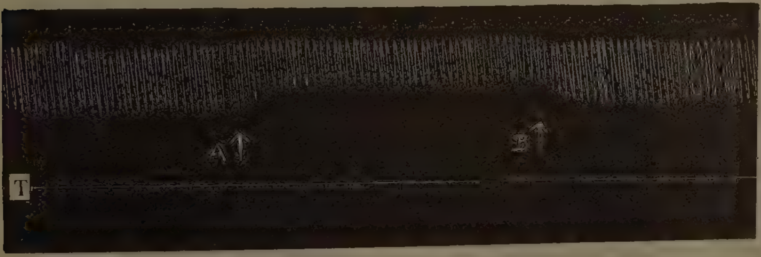

Fig. 2. A cardiogram of Panulirus with and without stimulation of the paired nerves. *A-B.*, Period of stimulation ; *T.*, Time line (2 seconds).

3. During the depressed phase of the membrane also the heart beat goes on uninterrupted.

4. The cardiogram obtained with and without stimulation of the paired nerves of the pericardial membrane is given in Fig. 2. That part of the cardiogram of the heart without stimulating the nerves of the pericardial membrane shows a greater amplitude, whereas the part on stimulation is distinctly restricted.

5. A tracing of the movements of the pericardial membrane is given in Fig. 3.

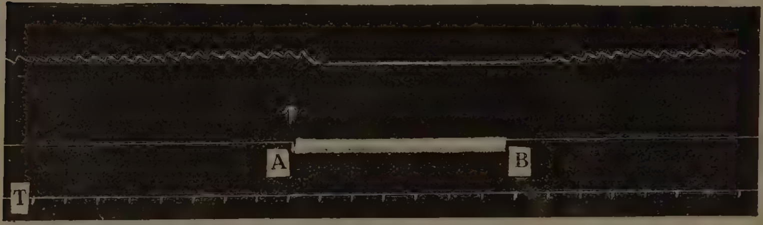

Fig. 3. A tracing of the movements of the pericardial membrane of Panulirus. *A-B.*, Period of stimulation ; *T.*, Time line (2 seconds).

6. When the heart is exposed by cutting through the carapace on the dorsal side, it could be seen that on contraction of the heart the blood flows out from it into the pericardial sinus instead of into the arteries.

Discussion

The part which the pericardial membrane plays in the circulation of crustacea does not seem to have been so far appreciated, and the only direct reference to it being that of Alexandrowicz (1932). Here we have shown that a well-developed nervous tract with five pairs of transverse nerves innervate the five pairs of pericardial muscles. Moreover, we have demonstrated that stimulation of these nerves brings about the depression of the paricardial membrane. Since the heart beat goes on uninterrupted after stimulation of the pericardial nerves, it can be deduced that the movements of the heart are independent of these nerves. Putting these facts together we explain the rôle of the pericardial membrane in circulation as follows. Since the heart beat is independant of the movement of the pericardial membrane, it may appear that the membrane may apparently play only an ancillary part in circulation as such. To understand the rôle of the pericardial membrane in circulation, let us suppose that it is not invested with muscles and does not play an active rôle. Even then circulation would have gone on as in insects, if the heart were to open directly into the sterno-visceral sinus. But in the crustacea the heart empties into the blood vessels and from them the blood reaches the sterno-visceral sinus. Then there are the gills and the branchio-pericardial channels intervening between the sternal sinus and the pericardial sinus. The pressure produced in the blood vessels as a result of contraction of the heart will be lost in the large sterno-visceral sinus and there will be no force left to let the blood into the gills and farther into the pericardial sinus. As the membrane is actively depressed by nervous action, it causes a diminution of pressure in the pericardial sinus on the one hand and an increase of pressure in the sterno-visceral sinus on the other. Thus it acts as a pressure pump in the sterno-visceral sinus and as a suction pump in the pericardial sinus. As the pressure at the termination of the arteries, is already high due to the contraction of the heart, the only direction the blood will be forced to flow from the sterno-visceral sinus is to the gills and from them into the pericardial sinus.

In summing up, it can be said that circulation is maintained at the present rate by the combined action of the heart and the pericardial membrane. The force-pump action of the heart drives blood into the arteries and from there into the sterno-visceral sinus. The force-pump action of the pericardial membrane driving blood from the sterno-visceral sinus into the gills and its suction-pump action whereby blood is drawn from the gills to the pericardial sinus, together complete the cycle and bring blood to the heart.

From the fact that blood passes out of the heart *via* the ostia into the pericardial sinus when pressure is reduced there, it follows that the requisite pressure to maintain a steady cardiac output into the arteries can be developed only if the membrane is lifted upwards. The maintainance of an optimum pressure in the pericardial sinus is thus an incidental additional rôle of the pericardial membrane.

Summary

1. The pericardial membrane is arched upwards and is tendinous in nature. It is invested on the ventral side with five pairs of symmetrically placed and very closely arranged striated muscle bands radiating towards the sides, leaving only a narrow space uncovered in the middle. When the muscles contract, they cause the depression of the membrane and when they relax the membrane resumes the arched original position.

2. The membrane is innervated by a network of nerves which are apparently extensions from the posterior pairs of the thin thoracic extensor nerves arising from the thoracic ganglion.

3. The heart contracts synchronously with the elevation of the pericardial membrane and relaxes along with its depression. The heart beat, however, is independent of the movement of the membrane.

4. When depressed the pericardial membrane forces the blood from the ventral sinus into the gills and at the same time it causes blood to be drawn into the pericardial sinus from the gills through the branchio-pericardial channels.

5. Without the maintenance of a high pressure in the pericardial sinus the ostia will not close tight so as to enable the heart to drive the blood into the arteries. This requisite high pressure is caused by the elevation of the pericardial membrane to its normal arched position.

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THE NATURE OF THE INTESTINAL PROTEINASE OF THE EARTHWORM, PHERETIMA ELONGATA (STEPH)

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THE proteolytic enzymes present in the digestive tract of earthworms have not been sufficiently studied and the nature and type of the earthworm proteinases are still imperfectly understood. Willem and Minne (1899), Lesser and Taschenberge (1908) and Winterstein (1911) were some of the earliest to report on the proteolytic activity of the intestinal secretion of the earthworms. More recently Millot (1945) has confirmed these reports. But no efforts were made to assess the properties of this enzyme. Hence this investigation was undertaken to obtain information on the properties of the protein-splitting enzyme from the intestine of a local earthworm, *Pheretima elongata* (Steph).

Material and Methods

Enzyme. The earthworms were well-washed and put in a basin of water for ten to twelve days. This not only cleaned them in and out but also tended to stimulate intestinal secretion. The animals were then gradually narcotized by using ethanol according to the method of Stephenson (1930). When they became inert, the intestine from segments XV-XXV was dissected out. These portions of intestine from a large number of earthworms were ground in a glass mortar together with a little amount of well-washed neutral sand. From this material a dry fat-free powder was prepared according to the method of Willstatter and Waldscmidt-Leitz (1921, 1923) by drying with acetone, acetone-ether and ether. This powder was stored in a desiccator in a refrigerator at 8-10° C.

The enzyme solution was prepared from this powder. For making the solution, a weighed portion of the powder was triturated with the extracting fluid—either a buffer solution, 50% glycerol or distilled water—till a uniform thin suspension was obtained. For each 5 g. powder a total of 100 ml. of extracting fluid was used. After the addition of a few drops of octyl alcohol, the suspension was kept agitated for about three hours. It was then centrifuged until a clear light-yellowish fluid was obtained.

All enzyme extracts were adjusted to pH, 8.7 before use. It was not found necessary to add enterokinase as activator.

Substrate. The substrate used to test the activity of the enzyme was casein purified from industrial casein adopting the method of Ray (1948). Stock solutions of it in buffer (pH 8.7) were kept in a refrigerator after adding a few drops of octyl alcohol.

Determination of proteinase activity. The method used was to estimate the initial rate of the first hydrolytic process *i.e.* the rate of disappearance of the protein in the initial stages. The method adopted was that of White and Bowman (1947) suitably modified.

This method is based on the colour produced by the interaction of the phenol reagent (Folin and Ciocalteu, 1927) with residual casein *i.e.* the casein that can be precipitated by trichloracetic acid after digestion for a particular period.

The procedures followed were based on those of White and Bowman except that all digestions were carried out at pH 8.7 using veronal acetate buffer of Michaelis (1931). While estimating, either the entire contents of the digestion vessel were used to obtain a single reading, several such vessels being used to establish a progressive curve, or successive samples were withdrawn from a single digestion mixture contained in an open trough.

Results

Effect of time on the rate of hydrolysis. Experiments carried out to find out whether there are any limiting factors with reference to the protein-liquefying activity of this enzyme by withdrawing samples at 0 time and subsequently at 5 min. intervals and estimating, showed (Fig. 1) that the rate of digestion was

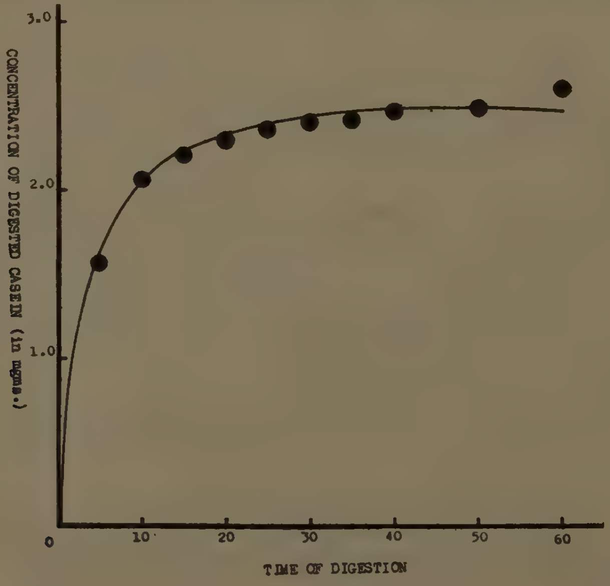

Fig. 1. Graph showing the effect of time on the rate of hydrolysis by the enzyme.

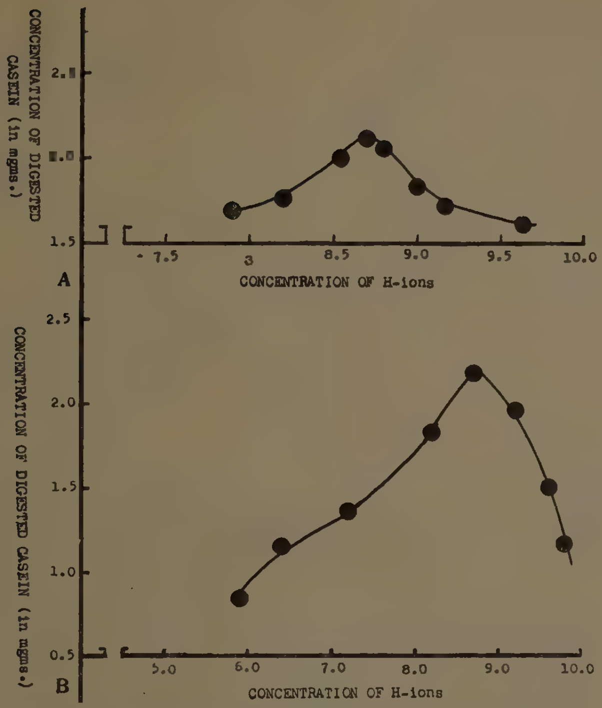

Fig. 2. *A*—Graph showing the effect of pH on the activity of the engyme.
B—Graph showing the effect of pH on the stability of the enzyme.

nearly proportional to the time of digestion for the first 15 minutes, after which there is a gradual decrease. This led to the adoption of 10 minutes' period for all the subsequent experiments.

Effect of pH. The optimum pH for this enzyme was found to be 8.7 when Michælis buffer was used. Fig. 2A shows the variation of the activity of the enzyme extract with pH of the medium. It shows that the enzyme still retained at least 75% activity at pH 7.9 and 9.6 indicating that it is tolerant of pH between 7.9 and 9.6.

Effect of pH on the stability of enzyme. When the activity of the samples of enzyme extract, which were held at different pH for 24 hours in refrigerator at 5°C. was estimated after bringing them to pH 8.7, it was noted (Fig. 2B) that the pH of maximum stability of the enzyme at 5°C. was in the region of the optimum pH of 8.7. It could also be made out that on the less alkaline side of the pH of storage,

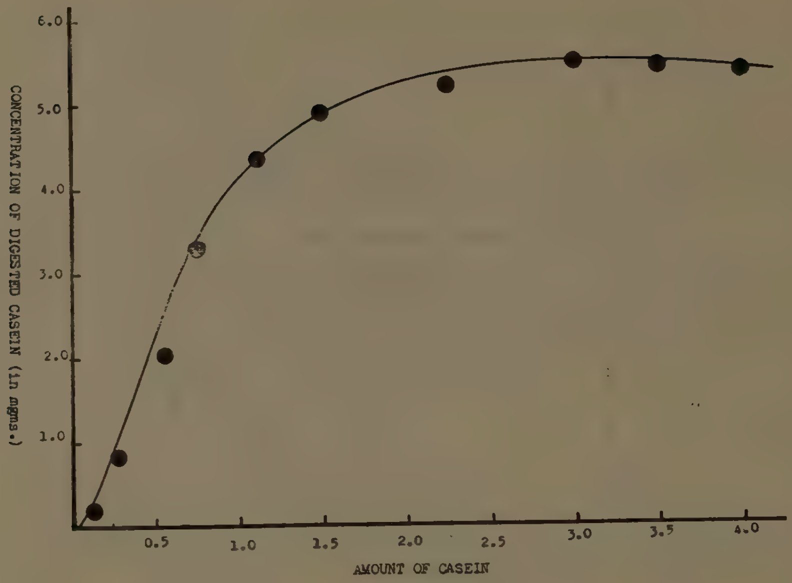

Fig. 3.—Graph showing the effect of substrate concentration on the activity of the enzyme.

the activity of the enzyme was lost rapidly, whereas on the alkaline side the loss was gradual,—a finding which was to be expected from the condition obtained in the previous experiment.

Effect of substrate concentration. Variation of activity with substrate concentration was measured by holding the concentration of the enzyme and all other conditions constant except the concentration of the substrate. It was found (Fig. 3) that the rate of the reaction increased with the rise in substrate concentration up to a certain maximum, but that with higher concentrations of the substrate no further increase in the rate was obtained.

Effect of enzyme concentration. On varying the concentration of the enzyme it was found (Fig. 4) that a nearly linear relationship existed between the amount of enzyme present and the initial velocity of hydrolysis over a fairly wide range. Such a relationship was to be expected on the basis of the theory of Michaelis and Menten (1913).

Heat inactivation. The effect of heat on activity of the enzyme was determined by heating the samples of the glycerol extract of the enzyme at pH 8·7 in the

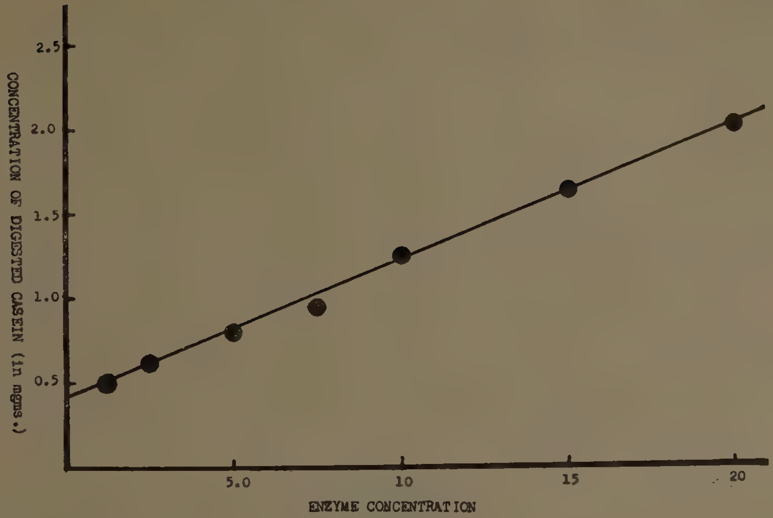

Fig. 4.—Graph showing the effect of the concentration of the enzyme on its activity.

absence of the substrate to a desired temperature for 5 minutes and then cooling them rapidly with crushed ice. The initial velocity of hydrolysis by the enzyme was then measured at 37°C.

The result (Fig. 5) showed that even at 40°C there was some inactivation of the enzyme. But on the other hand it was found that even after heat inactivation at as high a temperature as 80° more than 40% of the activity was retained. This showed that apparently this enzyme is heat resistant to some extent even at this high temperature.

Discussion

This study was undertaken to investigate the nature and type of the intestinal proteinase of the earthworm *Pheretima elongata* (Step). The experiments which have been made are far from complete, but they are sufficient to indicate the nature and type of the enzyme.

The results of this study shows that this proteinase hydrolyses casein optimally in the alkaline region *i. e.* like the tryptases it hydrolyses the cationic form of the proteins. It is further seen that in the alkaline region the enzyme is much more tolerant of pH than in the less alkaline region. The initial velocity of hydrolysis by this enzyme is quite rapid in the first 15 minutes and later on seems to fall off. Similar results have been reported by Farber and Wynne (1955) in the case of pancreatic trypsin.

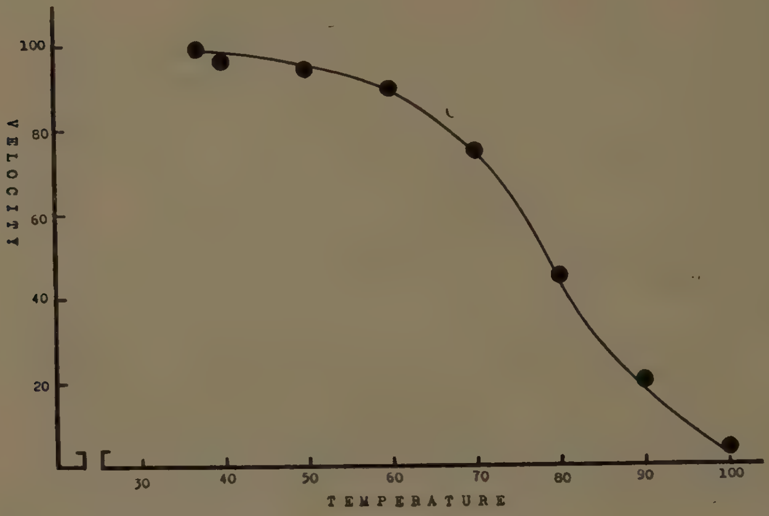

Fig. 5.—Graph showing the effect of temperature on the velocity of action of the enzyme.

That the action of an enzyme is proportional to the concentration of the substrate up to a point has been established by a number of workers. Similar results are found with this enzyme. The fact that a linear relationship between enzyme concentration and initial velocity of hydrolysis can be shown to be present even with a crude enzyme preparation that has been used here, indicates the absence of any concomitant inhibitors which, according to Northrop (1920) combine either with the enzyme or the substrate, thus blocking the enzymatic action. In addition, this enzyme like trypsin is found to be considerably heat resistant.

From these results it seems that out of the three main categories of proteinases, *i.e.* the pepsinases, tryptases and papainases, the evidence in favour of the intestinal proteinase of the earthworm belonging to the tryptic class of proteinases is the strongest. On the other hand this enzyme differs from tryptases in that it does not require to be activated by enterokinase, the specific activator of trypsin. It seems when this enzyme is obtained in the extract it is already in the fully active state.

Summary

1. Certain experiments were conducted to study the properties of the intestinal proteinase of the earthworm *Pheretima elongata* (Steph).

2. It is found that the amount of protein liquefied by the action of the enzyme increases with the time of digestion up to first 15 minutes, but after that the rate decreases considerably.

3. The optimum pH for this enzyme is 8·7 when Michælis buffer is used. The enzyme still shows at least 75% activity even at pH 7·9 and at 9·6, being much tolerant of pH in this range. This is further emphasised by the study of the effect of pH during storage on the stability of this enzyme.

4. With increased concentration of the substrate there is relative increase in the activity of the enzyme up to a particular maximum after which the rate of digestion becomes independent of the substrate concentration.

5. There is found to be a direct relationship between the concentration of the enzyme and the activity of the enzyme.

6. Like trypsin this enzyme is considerably heat resistant.

Acknowledgment

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PHYSIOLOGY OF EXCRETION IN THE COMMON INDIAN APPLE-SNAIL, *PILA GLOBOSA* (SWAINSON)*

Part I

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SINCE Strohl (1914) reviewed the excretory mechanism in Mollusca, Clementi (1914, 1915 and 1918), Baldwin and Needham (1934), Needham (1935), Picken (1937) and others contributed valuable though scattered data on the excretion of certain European marine, fresh-water and terrestrial forms e.g. *Helix pomatia, Anodonta cygnea.* But little work has so far been done to demonstrate the physicochemical processes involed in the nitrogen elimination and synthesis of urine by the nephridia of molluscs. The present investigations upon the common Indian apple-snail, *Pila globosa* were undertaken to elucidate the physiology of excretion in this snail. The results may also provide a bridge in the yet incomplete story of gastropod evolutionary radiations in different environments to some extent as *Pila globosa* is both a fresh-water and an amphibious form with a probable marine ancestry (Prashad 1932). This part deals chiefly with the occurrence and biosyntheses of uric acid as well as its elimination from the nephridium, while work on actual filtration in the formation of urine, osmo-regulation, etc. is in progress and will be published in the second part. The nephridium of Pila consists of an anterior and a posterior renal chamber and is in communication with the pericardium through the reno-pericardial opening (Fig. 1).

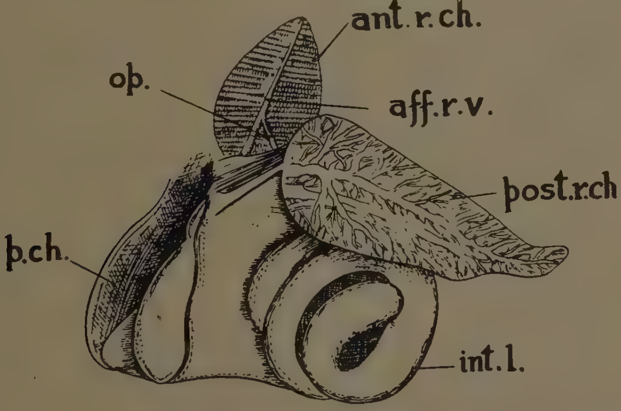

Fig. I. A diagram of the nephridium of *Pila globosa*. (Adapted from the diagram of Baini Prashad). (*The roof of the anterior renal chamber is removed*).

at.r.ch., anterior renal chamber ; aff.r.v., afferent renal vessel ; int.l., intestinal loop ; op., external opening of the anterior renal chamber into the pallial cavity seen from the inner side ; p.ch., pericardial chamber post.r.ch., posterior renal chamber.

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*(This work forms a part of the author's Ph.D. 'thesis')

Methods

It has been possible to assess nitrogen catabolism in animals partly by the analysis of excretory products and partly by the identification of enzymes responsible for the purine metabolism in the liver or hepatopancreas. Therefore a number of experiments were performed to estimate quantitatively the uric acid in the nephridium and the arginase and xanthine oxidase in the nephrdium as well as in the hepatopancreas of *Pila globosa* under different natural and experimental conditions. The uric acid was estimated in both the renal chambers in order to find out the rôle played by each of them in excretion. Besides this, a set of experiments was performed to determine the nature of excretion in the snail. The experiments were performed during the winter months of December and January in Northern India at which time of the year these snails usually æstivate, although a few were encountered without æstivating at the bottom of the ponds and the lakes as well.

In the first set of experiments æstivating animals were dug out from their burrows and their nephridia analysed immediately. In the second set, nephridia of æstivating animals were analysed after having maintained them in an aquarium in the laboratory for a period. The third set of analyses were made on nephridia of animals which were collected from ponds and lakes. In the second set, æstivating snails were washed off their adhering mud and each one of them was kept separately in a glass bowl with 200 ml. of distilled water. A few twigs of hydrilla were put in each bowl for the aeration of the water therein. The bowls were exposed to the sun for two hours daily. Each day the quantity of water in each bowl was checked and made up to the original volume if a bit of it had evaporated.

In the second set of experiments the two chambers of the nephridium of the snail were separately analysed. Similar estimations were carried out on the fifteenth day, on the twenty-first day, on the twenty-third day, on the twenty-fifth day and on the twenty-seventh day (Table III).

Estimation of Uric acid. The two renal chambers were separated and after the adhering body fluids were absorbed by a blotting-paper, each renal chamber was weighed in tarred crystal glasses in wet state and dried in a hot air-oven at 110°C. for about three hours till their weights became constant. The water content of each chamber was thereby calculated from these weighings. The dry tissue was powdered in a pestle and mortar with purified sand and the uric acid therein extracted repeatedly in distilled water on a steaming water bath at 80°C. The extract thus obtained was made up to the desired volume with distilled water. The uric acid in the aliquot samples of the extract was estimated by Bendict's Colorimetric method as described by Cole (1933).

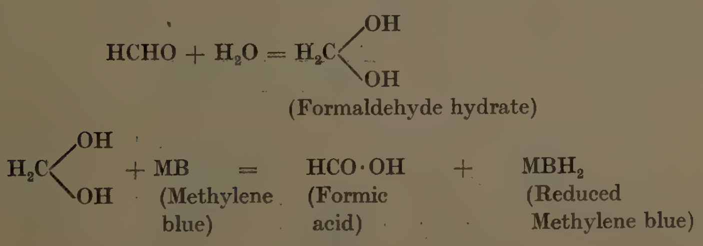
The extract was directly treated with arseno-phosphotungstate reagent and with 5 per cent sodium cyanide solution very carefully. The blue colour thus obtained was compared on a Klett-Bio-Colorimeter with that of a standard uric acid solution developed in the same way.

Estimation of Arginase. For the estimation of arginase the hepatopancreas and nephridium of æstivating and active *Pila globosa* from the aquarium were employed. The organ selected for analysis was dissected out from the snails in good physiological condition. Adherent tissue was removed as far as possible and surplus fluids were absorbed by a blotting-paper. The organs were weighed and ground thoroughly with purified sand in a chilled pestle and mortar. The 'brei' thus obtained was brought to pH 9·5 by the addition of very dilute alkali or acid and was incubated with a 5 per cent solution of l-arginine-monohydrochloride at 28°C for 60 minutes, using glycine solution as buffer. A few drops of toulene were addep as an antiseptic. The urea formed was decomposed into ammonia by ureas at pH 5 in the presence of acetate buffer. Ammonia being distilled off was estimated titrimetrically. The yield of urea-CO_2 c.m. was duly corrected for the tissue and solution blanks. All the results are expressed here in the QH notation of Krebs and Henseleit i.e. Ul urea-CO_2 per gram dry weight of tissue per 60 minutes, the temperature being 28°C. The activity so expressed being denoted by the symbol QH^{28} (Baldwin 1935).

Determination of Xanthine Oxidase. For the determination of xanthine oxidase, the nephridium and hepatopancreas of *Pila globosa* were employed. The 'brei' of the tissue was prepared as usual. Five ml. of supernatant tissue 'brei' was placed in a hard glass test tube with one ml. of 0·02 per cent methylene blue solution in distilled water and one ml. of 0·4 per cent formaldehyde solution. The contents of the tube were mixed by shaking and two ml. of a heavy mineral oil like paraffin in this case, was poured in the test tube to cover the contents in it. Controls and blanks were also run with boiled 'brei' and distilled water respectively in the same way. The tubes were kept in a water-bath maintained at 37°C. As soon as the contents of the tubes attained the temperature of the water-bath, the colour of the Methylene blue disappeared completely due to the reduction of the dye by the active enzyme present in the extract of the experiment, while the blue colour of the Methylene blue persisted in the blank and control. The experiments were repeated several times and it was confirmed that xanthine oxidase is present in the hepatopancreas and absent in the nephridium (Table IV).

The reaction is :—

(Sumner & Somers 1944)



$$HCHO + H_2O = H_2C\begin{matrix} OH \\ OH \end{matrix}$$

(Formaldehyde hydrate)

$$H_2C\begin{matrix} OH \\ OH \end{matrix} + \underset{\text{(Methylene blue)}}{MB} = \underset{\text{(Formic acid)}}{HCO\cdot OH} + \underset{\text{(Reduced Methylene blue)}}{MBH_2}$$

Results

The uric acid range as well as the water content of the nephridium in the æstivating, aquarium and lake specimens are given in Table I. The uric acid contents of the anterior and posterior chambers of the nephridium in the three series of snails—æstivating, aquarium and lake specimens—are separately given in Table II. The uric acid contents calculated per gram of dry weight of the anterior and posterior chambers of the nephridium obtained as a result of the six experiments involved in maintaining the snails in water-bowls for various periods after recovering them from æstivation are given in Table III. A graph depicting the excretion of uric acid during varying intervals of time is given in Fig. II. Table IV is a report on the hepatopancreas and nephridium of *Pila globosa* and *Achatina fulica* with reference to their uric acid, arginase and xanthine oxidase contents.

TABLE I

Water content and uric acid range in the nephridium of Pila globosa under different environmental conditions

Sl. No.	Environment	Percentage of water content		Uric acid in the nephridium mg./gm.	
		Anterior chamber	Posterior chamber	Minimum	Maximum
1.	Aestivating	78.6	85.4	83.96	241.74
2.	Aquarium	78.6	85.4	28.41	86.98
3.	Lake	78.6	85.4	Traces	15.16

TABLE II

Uric acid content in the anterior and posterior chambers of the nephridium of Pila globosa under different environmental conditions.

(In mg. per gm. dry weight of tissue)

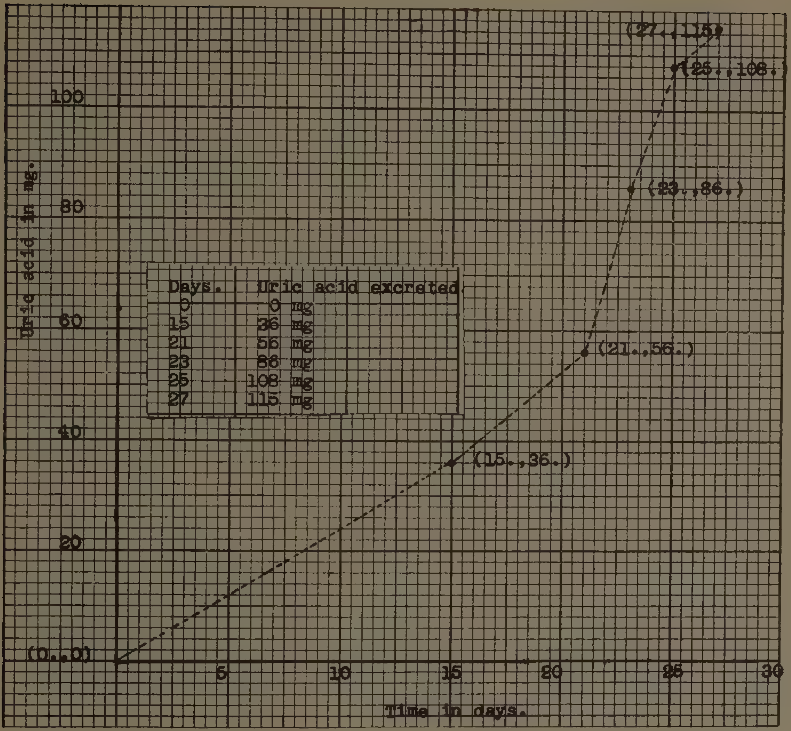
Sl. No.	Aestivating		Aquarium		Lake	
	Anterior chamber	Posterior chamber	Anterior chamber	Posterior chamber	Anterior chamber	Posterior chamber
1.	159.34	82.4	62.56	24.42	15.16	Traces
2. ...	78.3	30.88	59.65	7.31	7.89	Traces
3. ...	69.5	14.46	57.26	12.39	Traces	Nil
Average ...	102.38	42.58	59.82	14.64	7.68	Nil
Total ...	144.96		74.82		7.68	

TABLE III

Uric acid content in the anterior and posterior chambers of the nephridium in specimens of Pila globosa kept in water bowls for varying periods, after recovery from æstivation.

(In mg. per gm. dry weight of tissue)

Sl. No.	Period in water	Uric acid in nephridium		Total uric acid in nephridium
		Anterior chamber	Posterior chamber	
1.	Nil	102.38	42.58	144.96
2.	15 days	78.3	30.88	109.18
3.	21 days	79.04	10.23	89.27
4.	23 days	58.88	Traces	58.88
5.	25 days	37.09	Traces	37.09
6.	27 days	29.88	Nil	29.88



Days.	Uric acid excreted
0	0 mg
15	36 mg
21	56 mg
23	86 mg
25	108 mg
27	115 mg

FIG. II. A graph depicting the excretion of uric acid by the nephridium of *Pila globosa* during varying intervals of time (data derived from those given in Table III).

TABLE IV

Report on the hepatopancreas and nephridium of Pila globosa and Achatina fulica with reference to their uric acid, arginase and xanthine oxidase contents.

(Uric acid in mg. per gm. dry weight of tissue and arginase in QH notation of Krebs and Henseleit)

Animal	Part Examined	Uric acid	Arginase	Xanthine oxidase
Pila globosa (Swainson) ...	Hepatopancreas Nephridium	x 144.96	QH^{28} 50 Absent	Present Absent
Achatina fulica (Fer) ...	Hepatopancreas Nephridium	x 265	QH^{28} 315 x	x x

(Data taken from Lal and Saxena (1952) and Saxena (1953). X means not tested.

Discussion

The excretory function of the nephridium is borne out by the presence of appreciable amounts of uric acid therein. This has been found to vary in individual snails under different environmental conditions. In æstivating forms the uric acid content ranges from 83·96 mg. to 241·74 mg. per gm. dry weight of tissue, in aquarium living from 28·41 mg. to 86·98 mg. and in lake living ones from traces to 15·16 mg. (Table I). This striking gradient between the uric acid content of æstivating snails on the one hand and those of aquarium or those which have remained in an aquatic medium in nature on the other hand leads one to infer that in æstivating animals the uric acid is retained in the nephridium. This retention may be possibly made essential because the unfavourable conditions during extremes of temperature make the limitations of the available water very critical. When the snails are in water the elimination of excretory products may be facilitated by the current of water flushing through the body. With the æstivation the water-supply may be arrested and uricotelism, as indicated by the retention of uric acid in such an enormous quantity in the nephridium at such a juncture may obviate any chances of loss of water in excretion and a twofold toxæmia, uremia and ammonæmia.

According to Baldwin (1936) the day to day nuclein metabolism alone cannot be responsible for such a huge accumulation of uric acid in the nephridium of *Helix pomatia* and that it can only be accounted for by assuming that its mode of excretion is uricotelic. In view of the large amount of uric acid retained in the nephridium of *Pila globosa*, the same remark holds good in this case as well. Therefore uricotelism in *Pila globosa* is an adaptation to its terrestrial phase of life.

The presence of enzymes like ‘ arginase ’ and ‘ xanthine oxidase ’ in the hepatopancreas of *Pila globosa* as indicated in Table IV leads one to conclude that uricotelic metabolism and the possession of these enzymes are either independent or linked concomitants of terrestrial adaptations. But in view of the presence of ‘ arginase ’ and ‘ xanthine oxidase ’, the interesting possibility must not be lost sight of that the possible metabolic precursors of the uric acid in the nephridium may be ammonia, urea or tartonic acid. Wiener (1902) has even presented a possible scheme of the conversion of urea and tartonic acid into uric acid. Baldwin (1935) has also succeeded in demonstrating the conversion of urea into uric acid under the influence of the living slices of the hepatopancreas of *Helix pomatia.*

The genesis of this endogenous uric acid in the nephridium of *Pila globosa* may also be by the conversion of the purines, adenines and guanine to the intermediate hypoxanthine and of the latter to uric acid by means of xanthine oxidase. It can be concluded that both arginase and xanthine oxidase may be responsible for the genesis of the uric acid to some extent in *Pila globosa*, in the same way as Baldwin and Needham (1934 and 1935) related these enzymes to a possible synthesis of excretory products in *Helix pomatia.* The quantity of arginase in the hepatopancreas of *Pila globosa* is as low as QH^{28} 50 when compared with that of *Achatina fulica* having as much as QH^{28} 315 (Table IV). This indicates that arginase has to do something in the production of the uric acid in the nephridium because the uric acid in *Pila globosa* is only 144·96 mg. per gm. dry weight of tissue when compared with that of *Achatina fulica*, having as much as 265 mg. per gm. dry weight of tissue (Table IV). It is also interesting to note that so far it has not been possible to find any appreciable difference in the arginase content of the æstivating *Pila globosa* and those living in an aquarium (Lal & Saxena 1952). On the contrary no arginase was detected in those specimens of *Achatina fulica* which had hibernated for about six months in the laboratory (Saxena 1953).

The water content of the anterior and posterior renal chamber of *Pila globosa* is 78·6 and 85·4 per cent respectively. The results in Table II abundantly exhibit that the uric acid content of the anterior renal chamber of *Pila globosa* is greater than that of the posterior one in all animals, irrespective of their environmental conditions. In æstivating animals it is 102·38 mg. in the anterior and 42·58 mg. in the posterior renal chamber and falls to 59·82 mg. and 14·64 mg. respectively, in those which were maintained in the laboratory in an aquarium for one month; while in normal snails encountered from the natural habitat, it is only 7·68 mg. in the anterior and absent in the posterior renal chamber. Since in normally active snails no uric acid is present in the posterior renal chamber and small quantities are found in the anterior renal chamber, it is likely that the uric acid is passed into the anterior renal chamber. The anterior renal chamber communicates through the exterior renal opening in the pallial cavity whence the excretory products are discharged out (Fig. 1). When the snails undergo æstivation the uric acid may start accumulating in the anterior renal chamber as indicated by its large amount there (Table II). The presence of uric acid in the posterior renal chamber of the æstivating animals is likely on account of the fact that the anterior one after having retained maximum of uric acid cannot accommodate any more and instead it is passed on to the posterior one.

There is a regular decrease in the quantity of uric acid in the nephridium when snails recovered from æstivation are kept in water-bowls. This is borne out from results presented in Table III. The æstivating animals have a total of 144·96 mg. per gm. dry weight of the tissue in the entire nephridium. This figure falls after the animals have been kept in water and under conditions approaching those found in nature when the snail is most active. The snail which has remained in water for 15 days has 109·18 mg., after 21 days the quantity falls to 89·27 mg., after 23 days to 58·88 mg., after 25 days to 37·09 mg. and after 27 days to 29·88 mg. The gradual fall in uric acid quantity may be due to its elimination from the nephridium or conversion into some other non-toxic form, until finally after a period of 26 days the anterior chamber has only 29·88 mg. per gm. dry weight of the tissue and absent in the posterior one. The fall is, however, noticeable in both the chambers in all the experiments. The amount of uric acid supposed to be eliminated in a given period could not be recovered in the bowl except in traces, possibly because of sparing solubility of uric acid in the water at 20°C which was the temperature of the water.

It is evident from Figure II that the amount of uric acid, in mg. per gm. dry weight of the tissue in the nephridium is inversely proportional to the animal's stay in water. We can have a rough idea from this figure and Table III that the rate of elimination of uric acid in *Pila globosa* is fairly slow.

The uricotelism of *Pila globosa* is interesting in having a bearing on the question of phylogenetic reversibility in the evolution of this snail as a fresh-water amphibious form. Needham (1935) describes the uric acid content of the nephridium in marine operculates as very low; terrestrial snails on the other hand contain much uric acid. But the fresh-water species do not provide a clear cut series due to existing paucity of data on fresh-water snails as claimed by Prosser and others (1950). The operculate *Hydrobia jenkinsi* has little uric acid and has entered fresh-water relatively recently, whereas *Bythnia, Paludina* and *Limnaea stagnalis* contain much uric acid and probably have penetrated fresh-water secondarily. It appears that struggle for existence caused the migration of the ancestor of *Pila globosa* from its marine and estuarine ancestral abodes towards land, where water conservation forced them to adopt uricoteleic metabolism, and development within a cleidoic egg (Ranjah, 1922), and their penetration in fresh-water may be secondary.

Summary

1. When *Pila globosa* is in water the excretion of nitrogenous waste is facilitated by the current of water flushing through the body. With æstivation nitrogenous waste is stored as uric acid in order to conserve water and obiviate any chances of a twofold toxaemia, ammonæmia and uremia.

2. Uricotelism in *Pila globosa* is an adaption to the terrestrial phase of the snails' amphibious life.

3. Both arginase and xanthine oxidase in *Pila globosa* may be responsible for the biosynthesis of uric acid to some extent.

4. During æstivation uric acid is first retained in the anterior renal chamber and then in the posterior chamber in *Pila globosa.*

5. The rate of excretion in *Pila globosa* is fairly slow.

6. Penetration of *Pila globosa* in fresh-water appears to be secondary.

Acknowledgment

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THE NON-PROTEIN NITROGEN IN THE BLOOD OF SOME REPTILES AND MAMMALS.

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THE non-protein nitrogen (N.P.N.) of the blood is a collective concept which includes an assemblage of nitrogenous substances found in the protein-free filtrate of the blood. It includes substances like urea, uric acid, ammonia, creatine and creatinine, amino acids, glutathione and also minute quantities of substances of unknown nature. The majority of these substances are excretory products resulting from protein metabolism, on their way to the kidney to be eliminated from the body in the urine. On account of the toxicity of the substances the N. P. N. of the blood is normally maintained in any animal with only slight variations. In man this varies from 25-35 mg. per 100 cc. of blood with an average of about 30 mg. (Bodansky, 1938). However, dietary factors, diseases etc., have marked influence on the variation in the N. P. N. level of the blood.

Among the various N. P. N. constituents of the blood, urea forms the major part in mammals (45% in man—Hawk *el al.*, 1949). The urea level in the blood is highly variable depending on the amount of protein metabolism and also the rate of excretion (Bodansky—1938). In mammals urea is the main end product of protein metabolism, while in the majority of reptiles and all birds its place is taken up by uric acid.

Another major constituent of the N. P. N. of the blood—the amino acids, is derived from the dietary proteins. The concentration of it in the blood is found to vary according to the protein content of the ingested food. The amino acids of the blood form a source of nutrient material for the tissues and are found to be taken up by the tissues "with great avidity" (Folin, 1922).

Since the non-protein nitrogenous substances of the blood are organic crystalliods, they play a major rôle in maintaining the osmotic concentration of the blood. Thus in insects the amino acids, in elasmobranchs urea and in teleosts the trimethylamine oxide of the blood play major rôles in osmoregulation.

Even though much work has been done on the blood of vertebrates, only scant attention has been paid to the N. P. N. of the blood. Among the invertebrates Roeder (1953) has given extensive data regarding the N. P. N. content as well as the concentration of its different constituents in the blood of insects. Such a study has been found to throw a good deal of light on the nitrogen metabolism as well as on the physiological rôle of the blood in the insects.

Material and Methods

In the present investigation the N. P. N. content of the blood of the following animals was ascertained :—

Reptiles

(1) *Varanus griseus*
(2) *Ptyas mucosus*
(3) *Naja naja*
(4) *Vipera russelli*
(5) *Distira cyanocincta*
(6) *Natrix piscator*
(7) *Lissemys punctata*
(8) *Geomyda trijuga.*

Mammals

(1) *Suncus cæruleus*
(2) *Felis domesticus*
(3) *Pteropus giganteus*
(4) *Macaca radiata.*

A protein-free blood filtrate was prepared by the method of Folin and Wu as given by Hawk *et al* (1949). The blood was first laked by adding water. The proteins were then precipitated by the action of tungstic acid formed by the interaction of sodium tungstate and sulphuric acid.

The nitrogen content of the protein-free blood filtrate was estimated by the Micro-Kjeldhal method of Folin and Wu as described by Hawk *et al* (1949). A mixture of sulphuric acid and phosphoric acid was used for digestion. The ammonia produced was estimated by directly nesselerizing the digested solution and comparing the colour produced with that of a standard containing a known quantity of ammonia in the Klett-Summerson photo-electric colorimeter.

Results

The table given below shows the results obtained for N.P.N. content of the blood of the animals examined. The N.P.N. values for some mammals collected from previous works are also given for comparison.

TABLE I

N. P. N. Content of the blood of some reptiles and mammals.

Animal	N. P. N. Content in mg./100 ml. of blood
Reptiles	
1. Varanus	90.585 ± 9.006
2. Lissemys	43.549 ± 4.839
3. Geomyda	74.758 ± 10.242
4. Ptyas	99.364 ± 16.031
5. Vipera	45.763 ± 6.356
6. Naja	95.150 ± 6.167
7. Distira	110.848 ± 9.153
8. Natrix	75.455 ± 31.743
Mammals	
1. Suncus	111.307 ± 24.542
2. Pteropus	42.909 ± 14.784
3. Felis	38.903 ± 16.912
4. Macaca	47.314 ± 23.797

TABLE II

N. P. N. Values for some mammals collected from previous work.

Animal	Amount of N. P. N. in mg. per 100 ml. of blood	Reference
Rat	54 ± 0.8	Leathem 1948
Man	37.7 to 55.0	Folin 1922
Man	18 to 45	Chakravarti and Mitra 1951

Discussion

An examination of the N. P. N. values of the blood of the various animals investigated shows that there is considerable variation among the members of reptiles as well as among the mammals. Thus Vipera, a snake, shows a value of

45.763±6.356, while Distira, another snake, shows 110.848±9.153. In mammals Suncus has a value of 111.307±24.542, while that of Felis is only 38.903±16.912.

The values available from previous work (Table II) for rats and for man compare favourably with those obtained by me for Felis, Pteropus and Macaca (Table–I)

Compared to Felis, Pteropus and Macaca, Suncus shows a very high N. P. N. content in the blood (Table I). For an explanation for the high value of N. P. N. in Suncus we are tempted to suggest the following. As the N. P. N. content of the blood closely follows the rate of protein metabolism, nitrogen excretion and dietary factors, an explanation for the high N. P. N. content of the blood of Suncus may be sought among the undermentioned factors. Due to their smaller size, their metabolic rate is very high and consequently, as is the case with such small animals, they feed voraciously consuming nearly their own weight of food in 24 hours (Prosser *et al.*, 1950). As they belong to the order Insectivora, their food consists of insects. Not only that an insect diet consists of a large quantity of proteins, but also the body fluid of insects contains a high percentage of uric acid and amino acids (Roeder 1953). These substances are ingested in large quantities by the animal in its food. A good proportion of the exogenous uric acid and amino acids taken in the food might find its way into the blood regularly, besides a large quantity of endogenous N. P. N. resulting from the catabolism of proteins. In comparison with this, Pteropus and Macaca are vegetarians, while Felis though a carnivore has become omnivorous under domestication. Therefore the comparatively low N. P. N. content of the blood of these animals may be due to their proportionately low protein diet and consequent low protein metabolism.

The analytical data obtained regarding the amino acid content of the various animals show that even though Suncus has a high amino acid content in the blood (14.138±4.556) compared to those of Felis (10.323±0.441), Pteropus (9.043±1.043) and Macaca (7.070±0.237), (S. G. Nair, unpublished) quantitatively it is not sufficient to account for the high N. P. N. content in its blood. Hence, a more detailed study alone will reveal as to which particular constituent is responsible for this increased N. P. N. content in the blood of this animal, and its significance.

In the case of reptiles too the range of variation is great with high values in several cases. Any conclusions from these figures are bound to be premature.

Summary.

1. There is a considerable variation in the N. P. N. content of the blood among the members in reptiles as well as in mammals.

2. The high N. P. N. value obtained for the blood of Suncus among the mammals is explained as due to its small size and consequent high metabolic rate and to its feeding habits—namely that of ingesting a large quantity of insects.

3. Since the amount of non-protein nitrogen is dependent on several sources, explanations concerning the variations within any group are bound to be premature.

Acknowledgment

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THE NUTRITIVE CAPACITY OF THE BLOOD OF SOME REPRESENTATIVE CRUSTACEANS AND MOLLUSCS

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IN addition to its rôle as a carrier of oxygen, the blood has also a nutritive function. Absorption and transport of the digested food are aspects implied in the nutritive capacity of the blood. Since immediately after digestion, the blood is enriched with the products of digestion, viz.: glucose, fatty acids and glycerine, as well as amino-acids, the composition of the blood will be varying in the same animal. Variation in this respect occurs also amongst the members of the same species as well as amongst members of different species. Yet, there seems to be limits of range in the composition of blood in the different classes of animals.

The blood sugar level of the lower invertebrates is very low. The majority of invertebrates show no mechanism for the regulation of blood sugar level (Florkin, 1949). However, Roche and Dumazert (1949) have reported the occurrence of about 22 mgm. per cent of glucose in the blood of the crab *Cancer pagurus* and prolonged fasting for one month is reported to have not reduced its blood sugar level.

As regards the lipid contents of the blood in crustaceans and molluscs, very little information is available. A definite increase in the amino-nitrogen of the blood during digestion is known. That the formation of proteins of different types is dependent on the availability of the requisite amino-acids which go into their composition, is also known. The knowledge about the amino acids occurring in the blood of crustaceans and molluscs is scanty indeed.

In this paper the glucose and fat contents of the blood of a few local crustaceans and molluscs are reported. A chromatographic detection of free amino-acids met with in their blood is also given. The animals worked out are among crustaceans *Panulirus polyphagus*, *Scylla serrata*, *Paratelphusa guerini*, and among molluscs *Pila globosa*, *Telescopium telescopium*, Aplysia sp., *Lamellidens lamellidens*, *Paphia malabarica*, and Octopus sp., Investigations on amino acid contents of the blood have, however, been carried out only in *Panulirus polyphagus, Scylla serrata and Telescopium telescopium.*

Methods

The method of Folin aud Wu (1920) has been employed for the determination of blood glucose. The protein-freed blood filtrate is heated with alkaline copper solution, using a special tube for preventing reoxidation. When the cuprous oxide formed is treated with a solution of phosphomolybdic acid, a blue colour is produced. This is compared with a standard in a colorimeter.

Fat has been extracted from the whole blood by the ordinary ether extraction method, after drying it on sodium sulphate for ten minutes at 100°C. in an air oven.

For the detection of free amino acids in the blood, it is first pipetted in three times its volume of absolute alcohol. This process precipitates the proteins and the free amino acids are left intact. After centrifuging, the protein-free alcoholic extract is mixed with three times its volume of chloroform. As a result an aqueous layer is formed on top of the chloroform-alcohol mixture. The amino acids present in the aqueous solution are separated and identified using Rutter's technique (1950) of circular chromatography as modified by Giri and Rao (1952).

Results

The following table gives the glucose and fat content as well as the free amino acids met with in the blood of the animals tested.

Animal	Glucose in mgm. per 100 c.c. of blood	Fat in gm. per 100 c.c. of blood	Free amino acids
Panulirus ...	13.63 —58.064	0.2	Glycine, serine, arginine, histidine, lysine, alanine, glutamic acid and threonine.
Scylla ...	11.76 —161	0.15—0.21	All the above except glutamic acid and threonine and in addition proline.
Paratelphusa ...	10.576—57.7	0.338	......
Pila ...	9.574—12.162	0.62—0.69	......
Telescopium ...	4.411—25	0.295	All listed above for Panulirus except glutamic acid and alanine, and in addition leucine, methionine and valine.
Aplysia ...	5.923— 7.312	0.198	......
Lamellidens ...	20.512—21.794	0.012	......
Paphia ...	3.5 — 4.312	0.01	......
Octopus ...	11.485—12.25	0.25	......

Discussion

A comparison of the glucemic levels in the various animals examined shows the possibility of a very wide range of variation. Thus in the crustaceans it varies between 10·576 to 161 mgm. per 100 c.c. of blood and in the molluscs between 3·5 to 21. 794 mgm. per 100 c.c. of blood. It is hazardous to generalise and draw any type of conclusion regarding the glucemic levels in the blood of these groups of animals only with the data available here.

It is surprising, however, that the crustaceans Panulirus, Paratelphusa and Scylla can tolerate a large amount of glucose in their blood. This is in addition to the high concentration of sodium chloride present in the first and third. It is known that in man the introduction of even 1 per cent salt solution into the blood stream acts as a glycuretic and causes a decided increase in sugar in the urine. In this connection the suggestion of Myers (1920) that the relatively large amount of calcium salts in the blood of invertebrates may lessen the permeability of the excretory membranes. It may therefore be on account of the large amount of calcium in the blood that these crustaceans are able to maintain such high percentages of sugar in their blood.

The lipid content of the blood also shows considerable variation. Thus it is only 0·01 gm. per 100 c.c. in Paphia and 0·62 to 0·69 gm. in Pila. Since both glucose and fat are utilized as fuel in the animal body, the presence of more of one of them may mean less amount of the other. From a comparison of crustaceans with molluscs in this respect, it can be seen that in the latter group very low levels of fat may occur as in Paphia and Lamellidens. It may also be possible that the fat percentage is very low in the blood of lamellibranchs.

The number of free amino acids met with in the blood in these groups is surprisingly small. Glycine, serine, arginine, histidine and lysine are present in all the three animals. Alanine is present in the blood of Panulirus and Scylla, but absent in the blood in Telescopium. Leucine is present in Telescopium, but absent in the other two. Similarly proline is present in Scylla, but absent in the other two. Telescopium alone shows methionine and valine. Glutamic acid is present only in Panulirus.

Summary

1. Taking the amounts of glucose and fat as indicative of the nutritive capacity of blood, their respective amounts have been determined in a few local crustaceans and molluscs.

2. The crustaceans seem to be capable of tolerating a large amount of glucose in their blood. It may be due to the large amount of calcium met with in the blood of these animals.

3. Considerable variation occurs in the amount of both glucose and fat met with in the blood of both crustaceans and molluscs.

4. The lamellibranchs Lamellidens and Paphia show only insignificant amounts of fat in their blood. The corresponding figures for the other animals are very much higher.

5. The number of free amino acids met with in the blood of the animals examined is small. Differences in their assortment are mentioned.

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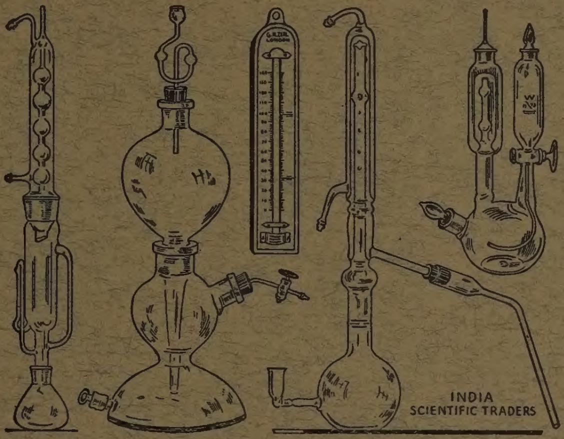

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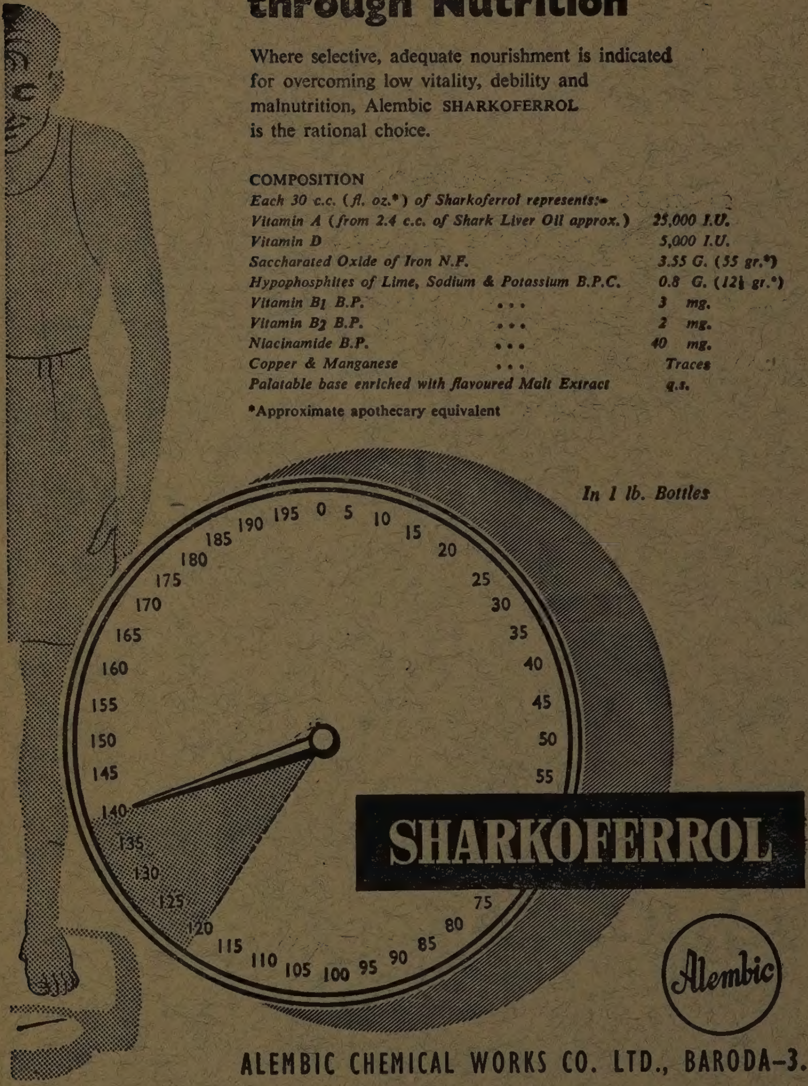

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